

# THE CLEMATIS FREMONTII VAR. RIEHLII POPULATION IN THE OZARKS<sup>1</sup>

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## INTRODUCTION

During the latter half of the nineteenth century, the Darwinian theory of evolution by natural selection inspired a vast amount of research which was largely directed toward tracing of phylogenies and demonstrating the adaptation of organisms to their environment. However, the theory has recently been somewhat out of fashion. Its abeyance was coincident with the rise after 1900 of the new science of genetics and its companion, modern nuclear cytology. Preoccupation with the new disciplines partly accounted for the neglect of evolutionary studies. But it was partly due to the fact that the new principles which were emerging, the particulate theory of inheritance and the DeVriesian mutation theory, seemed to contradict some of the premises of Darwinism.

To-day there is a resurgence of interest in evolutionary matters. It is apparent that Darwin's theory, in its essentials, still stands. Modern genetics throws immediate light on some points which were hidden to Darwin. Gene mutation, which has now been studied in the laboratory and in the field, is seen to be the source of the omnipresent variation which Darwin pointed out but did not explain. The particulate nature of inheritance, far from being contradictory to the theory of natural selection, has been shown by Fisher ('30) to be essential to evolutionary change. A mathematical theory has been constructed, largely by Wright (for bibliography and non-mathematical summary, see Dobzhansky, '41), which permits rates of change of gene frequency to be calculated from mutation rates, the selective advantage of one gene over another, and size of population, under various systems of mating. These changes in gene frequency, when integrated for the entire genotype of the organism and over its entire population, may be said to constitute the primary steps in evolution.

The most important generalizations which Wright has made from his mathematical studies are those relating to the effect of population size, or more accurately, of what he terms "population number," upon the rate and course of evolution. In a very large, freely interbreeding population, where the number of potential mates for each breeding individual is large in relation to mutation rates, selection is strongly operative. The genotypes of the organism will then tend to cluster closely about a peak in the surface of adaptive values. The organism will be well adapted to its environment, but its over-all variability will be somewhat

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restricted. It will adapt itself to a secular change in the environment by moving to a new adaptive peak, but it will not be able to cross an adaptive valley to reach a conceivably higher peak. In a very small population, or in one which is divided into small isolated colonies, the range of variation will be restricted locally, though there may be considerable variation from one colony to another. The phenomenon of "genetic drift" will come into play. There will be a random loss and fixation of genes resulting from the errors of sampling of the gametes which reproduce each generation, largely without regard to the adaptive value of the genes involved. As a result, the fate of an organism which is too greatly restricted in numbers is extinction. Wright considers the most favorable condition for continuing evolution to be that of a large population broken up into numerous small colonies which are connected by occasional migration. Each colony will be free to explore the field of gene combinations without the restrictive effect of too rigid selection. Differentiation within the population will be largely non-adaptive, but some of the colonies will be expected to arrive at favorable genotypes or adaptive peaks, perhaps quite different from the original one about which the population centered. Such colonies will tend to increase in numbers and to bring the remainder of the population up to their genotype through migration. This combination of non-adaptive differentiation of partially isolated local groups with intergroup selection will permit evolutionary advance without a secular change in conditions.

Wright's theory has become an important part of modern evolutionary thought. Eventually it may have the same importance and validity in the field of evolution which the publications of J. Willard Gibbs have in chemical thermodynamics. However, it is merely a theory, and it is impossible at present to judge whether it adequately accounts for evolutionary changes which are known to take place. It urgently requires testing against facts from the field. The facts required for its examination, or the examination of any other theory which attempts to explain the mechanism of evolutionary change, are of many kinds. The beauty of Wright's theory is that it indicates clearly the kinds of information which are important. Detailed information is required about life histories of various organisms, particularly the details of reproduction. Data are required on the numbers of individuals, and on their pattern of distribution, both at present and over a span of years. The pattern of differentiation must be understood in detail. Detailed information about sources of evolutionary change such as mutation, hybridization, and chromosomal changes must be obtained. Furthermore, the data on all these points must be coordinated for individual organisms. Such a body of detailed and coordinated facts scarcely exists for any organism, but is of first importance in any discussion of evolution.

The present study of *Clematis Fremontii* var. *Rieblii* was undertaken with the object of working out a picture of the features of its population structure which are of evolutionary importance, and if possible of making an estimate of evolutionary trends within the population. The pattern of distribution has been worked



out in some detail. Biological factors such as method of pollination, seed dissemination, seed germination and longevity have been examined. Variation in flower and leaf characters has been studied. An attempt has been made to obtain quantitative data where possible, but many of the present conclusions are based on subjective judgment; the difficulties are many.

*Clematis Fremontii* var. *Rieblii* is a member of the section VIORNA, subsection INTEGRIFOLIAE of *Clematis* (Erickson, '43a). Besides the Eurasian *C. integrifolia*, which probably should be placed in the subsection, it includes four closely related species and one or two varieties. They are comparatively well-marked and uniform entities, contrasting with such polymorphic species as *C. Pitcheri*. All except *C. ochroleuca* are of restricted distribution, characteristically occurring on rocky barrens. *C. albicoma* and the recently proposed *C. albicoma* var. *coactilis* (Fernald, '43) occur on the Devonian shale barrens of the Appalachians of West Virginia and Virginia (Wherry, '30, '31). *C. viticaulis*, also a shale barren plant, has been collected at a single locality. *C. Fremontii* is a secondary species in the *Andropogon scoparius* habitat of the mixed prairie of north-central Kansas. There it is usually limited to the upper slopes above the brows of hills where there is an outcrop of Fort Hays Limestone or Smoky Hill Chalk (Albertson, '37, '42).

*C. Fremontii* var. *Rieblii* is restricted to an area of somewhat more than 400 sq. mi. in Jefferson County and portions of two adjacent counties in east-central Missouri. A distribution map and a discussion of the limits of its distribution have been published (Erickson, '43b). The plant is wholly restricted to glades, rocky barrens which occur on south- and west-facing slopes of otherwise wooded ridges. The glades occur on outcrops of the thin-bedded dolomite of several formations of the Canadian Series, particularly the Cotter and Powell. Their distribution follows the outcrop belt of these formations; glades and the similar bald knobs of south-central Missouri encircle the Ozark dome. On a smaller scale, their occurrence is determined by the presence of sufficient local relief in conjunction with the outcrop of thin-bedded dolomite. They are characterized by a thin soil cover, which is slightly acid and fairly high in organic matter, and by an extreme set of environmental conditions: saturation to the point of seepage in late fall and early spring, and desiccation during the summer months (Erickson, Brenner and Wraight, '42). The glade habitat appears to be an edaphic climax, rather than a stage in the succession to upland forest, or a product of a biotic influence, such as grazing by cattle. The red cedar, *Juniperus virginiana*, is the most characteristic tree associated with the glades; the glades can be recognized from a distance by the contrast which the dark green of the cedars offers to the surrounding broad-leaved forest. The red cedars occasionally form an open cover, but usually occur as scattered individuals and may even be absent. The dominant plant is clearly the bluestem, *Andropogon scoparius*, though there are other grasses, and several other species make a conspicuous seasonal show of flowers, such as *Leavenworthia uniflora*, *Houstonia angustifolia*, and particularly, *Rudbeckia missouriensis*. Many of the plants have xeromorphic characteristics. Flor-



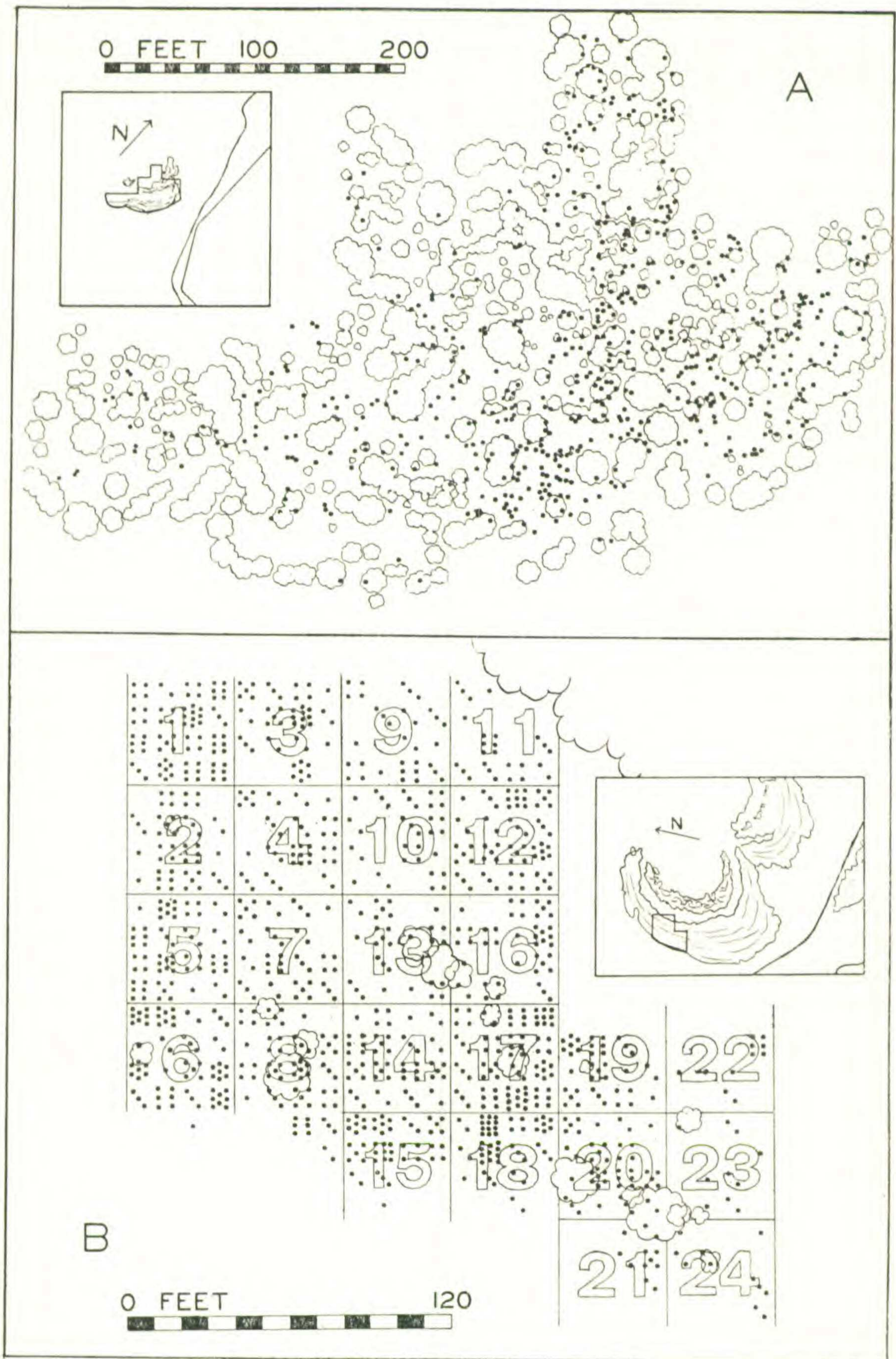


Fig. 1. Distribution of *C. Fremontii* var. *Rieblii* on greater part of small glade at R.2E, T.42N, S.10C (A); and on portion of larger glade at R.6E, T.39N, S.4-0 (B). Inserts show relation of area studied to glade as a whole. Small black dots represent *Clematis* plants, irregular outlines, trees. Domino effect in B is due to the fact that plants were counted in each 10-ft. quadrat, not plotted as they were for A. Numbered 40-ft. quadrats are referred to on p. 443 et seq.



istically, the glades are related to the shale barrens of the Appalachians (Wherry, '30), to the cedar glades of the Nashville Basin in Tennessee (Freeman, '33), to portions of the prairies of Kansas and Nebraska (Albertson, '37), and to glade-like grassy areas in the Arbuckle Mountains of Oklahoma and the Edwards Plateau of Texas.

#### DISTRIBUTION PATTERN

Because of its striking appearance, *Clematis Fremontii* var. *RiehlII* is a conspicuous member of the glade community, but in numbers it is subordinate. Its distribution on a number of glades has been studied in some detail. Figure 1 shows the distribution on the greater part of a small glade and a portion of a larger one that are not so much representative as illustrating approximately two extreme situations in which the plant is found. The maps were prepared from data obtained by laying out 10-ft. quadrats on the glades and plotting or counting the plants in each quadrat. On several other glades (fig. 2) the distribution has been studied by laying out 10-ft. transects of contiguous 10-ft. quadrats, usually at 250-ft. (50-pace) intervals, and normal to the "contour lines" formed by outcropping rock ledges.

Erickson and Stehn ('45) have published a statistical analysis of these data. They have pointed out that the data cannot be regarded as representing random (Poisson) distributions. Field observations suggested that the departure from randomness has its basis in a lack of uniformity of different portions of the glades as a habitat for *Clematis*. The data have been fitted by calculating two Poisson distributions for each glade, an "economic distribution," corresponding to suitable portions of the glade, and an "adventitious distribution," whose mean is small, representing unsuitable portions. The mean of the former is regarded as equivalent to Elton's ('32, '33) economic density.

The results of this statistical analysis should be considered in the light of field observations of conditions on the glades. The density counts are summarized in Table I. As contrasted with the tenfold variation in uncorrected, mean density,  $m_0$ , the economic densities,  $m_1$ , show a better agreement. The economic means of 1.02 and 1.10 plants per 100 sq. ft. are both for small glades; the rest, with means clustering around three or four plants per 100 sq. ft., apply to larger glades. The small glade at R.2E, T.42N, S.10C ( $m_1 = 1.02$ ) is remarkable for its inaccessibility, and for the large size and number of red cedars. The data of line 6 in Table I ( $m_1 = 1.10$ ) were obtained by combining data from two similar, adjacent glades in R.5E, T.40N, S.13. Both are small glades, though without such a conspicuous cover of red cedars as glade No. 1. The fact that the data appear to fall into two groups on the basis of economic density values is a reflection of the tendency, not recognized in the earlier field work, to select the larger, more "typical" glades for study. If more representative data were at hand, it would probably be found that the economic density is somewhat a function of the size of the glade, reaching an optimum value of three to four plants per 100 sq. ft. on large glades, and being smaller on smaller glades. It is thought that the conditions



TABLE I  
 DISTRIBUTION OF *CLEMATIS* ON GLADES  
 IN FRANKLIN CO. AND JEFFERSON CO., MO.  
 (Glade numbers correspond with those of fig. 2)

No.	Location	Area (acres)	Est. number of <i>Clematis</i>	Mean density ( $m_0$ )	Economic density ( $m_1$ )
1	R. 2E, T. 42N, S. 10C	2.8	1,140	0.57	1.02
2	R. 2E, T. 42N, S. 15B	13.4	6,780	1.16	3.61
3	R. 3E, T. 41N, S. 25	23.3	3,880	0.41	2.98
4	R. 4E, T. 40N, S. 15B	14.9	5,580	0.86	3.87
5	R. 4E, T. 40N, S. 15D	14.8	13,230	2.05	4.07
6	R. 5E, T. 40N, S. 13A	5.4	830	0.34	1.10
7	R. 5E, T. 40N, S. 13E	20.7	32,000	3.69	2.51
8	R. 6E, T. 39N, S. 4-0	24.3	11,300	2.85	3.76

of winter saturation and summer desiccation, etc., referred to by Erickson, Brenner and Wraight ('42), are developed to the extreme only on the largest glades, being somewhat ameliorated on smaller glades more closely surrounded by forest. *Clematis* may be limited to glades not because of its special adaptation to their physical environment, but because it finds competition from other species too severe elsewhere, as Salisbury ('29) has found to be the case for other plants of barrens, such as *Ranunculus parviflorus*. If that is so, *Clematis* would be expected to reach its optimum density on the large glades where biological competition is presumably least severe.

The implication of this statistical treatment, that the glades can be divided into two portions on the basis of their suitability for *Clematis*, deserves some amplification. A prominent physical characteristic of the glades is the occurrence at intervals of parallel outcrops of more massive rock than the thin-bedded dolomite which forms the glade proper. On the aerial photographs of the region, which were studied as a preliminary to the field work, these outcrops give the appearance of contour lines, and aid greatly in recognition of the glades. In the field the ledges are found to vary greatly in distinctness. *Clematis* characteristically occurs just below such a ledge of rock, though it is by no means strictly limited to such places. This and the fact that it seems to be more abundant near the lower edge of a glade suggest that one of the factors determining its presence is the amount of seepage water available during the spring. The unsuitable portions of the glade, or "blanks," are of at least three kinds: the exceedingly barren areas just above a ledge of massive rock, which are strewn with chert fragments and occupied almost exclusively by a sparse growth of the small grass, *Sporobolus*



*beterolepis*; very grassy portions, where *Clematis* would presumably meet severe competition with *Andropogon*; and small clusters of trees, *Juniperus virginiana*, *Bumelia lanuginosa*, *Cornus florida*, etc., which occur at intervals on the glades, often where a gully has developed.

It is apparent then that the distribution of *C. Fremontii* var. *Rieblii* on individual glades is characterized by considerable aggregation. The *aggregates* of plants are not well delimited, as can be seen by reference to fig. 1, but they do exist. They vary considerably in area, and they may include a few plants to a few hundred.

While the aggregates of plants are undoubtedly important in breaking up the population into local groups, the *glades* themselves, by their greater definiteness of outline and more complete isolation, must also be significant. On the distribution map (Erickson, '43b, fig. 2), about 15 negative records were plotted within the distribution area of the *Clematis*, with 160-odd positive records, indicating that roughly 87 per cent of the glades support some plants. Furthermore, the plant has never been found except on a glade, and it is probably justifiable, as a first approximation, to regard glades and *colonies* of plants as equivalent in examining the organization of the population. Those which have been carefully studied (Table I) vary in area from 2.8 to 24.3 acres, and in estimated number of plants from 830 to 32,000. However, it has been pointed out above that the sampling involved has not been satisfactory. Between 200 and 250 glades have been visited more briefly, and some impressions gained from that experience should be pertinent. The glades vary in area from about 80 acres (large glade two miles north of Plattin) to small grassy areas which scarcely merit the name. In R.3E, T.41N, S.1-18, the total area in glades was measured by placing the tracings of the aerial photographs over a piece of paper ruled in small squares and counting the squares covered by glade outlines. Sixty glades were counted with a total area of 123 acres. Here, then, the average glade measures very nearly two acres in area. The number of plants per glade varies greatly, and probably corresponds only roughly with the area of the glade. Several glades of considerable area have been visited on which only one or a very few plants could be found. The upper limit in size of a colony is indicated by the figures in the fourth column of Table I, and the average size of a colony appears to be about 970, as calculated on page 422.

An impression of the degree of isolation between separate glades (= colonies) can be gained by examining fig. 2 and the larger scale map (fig. 3). On the whole, there is little difficulty in defining separate glades. It is apparent that the glades are not randomly distributed. No attempt has been made to treat this matter statistically, but obvious relations of the glades to the drainage pattern can be seen, as, for example, at R.4E, T.40N, S.11 and 14 (fig. 3) where the glades are ranged on either side of "branches" of Cotter Creek. Such topographically determined *clusters* of glades must also have significance in the subdivision of the population into local groups.



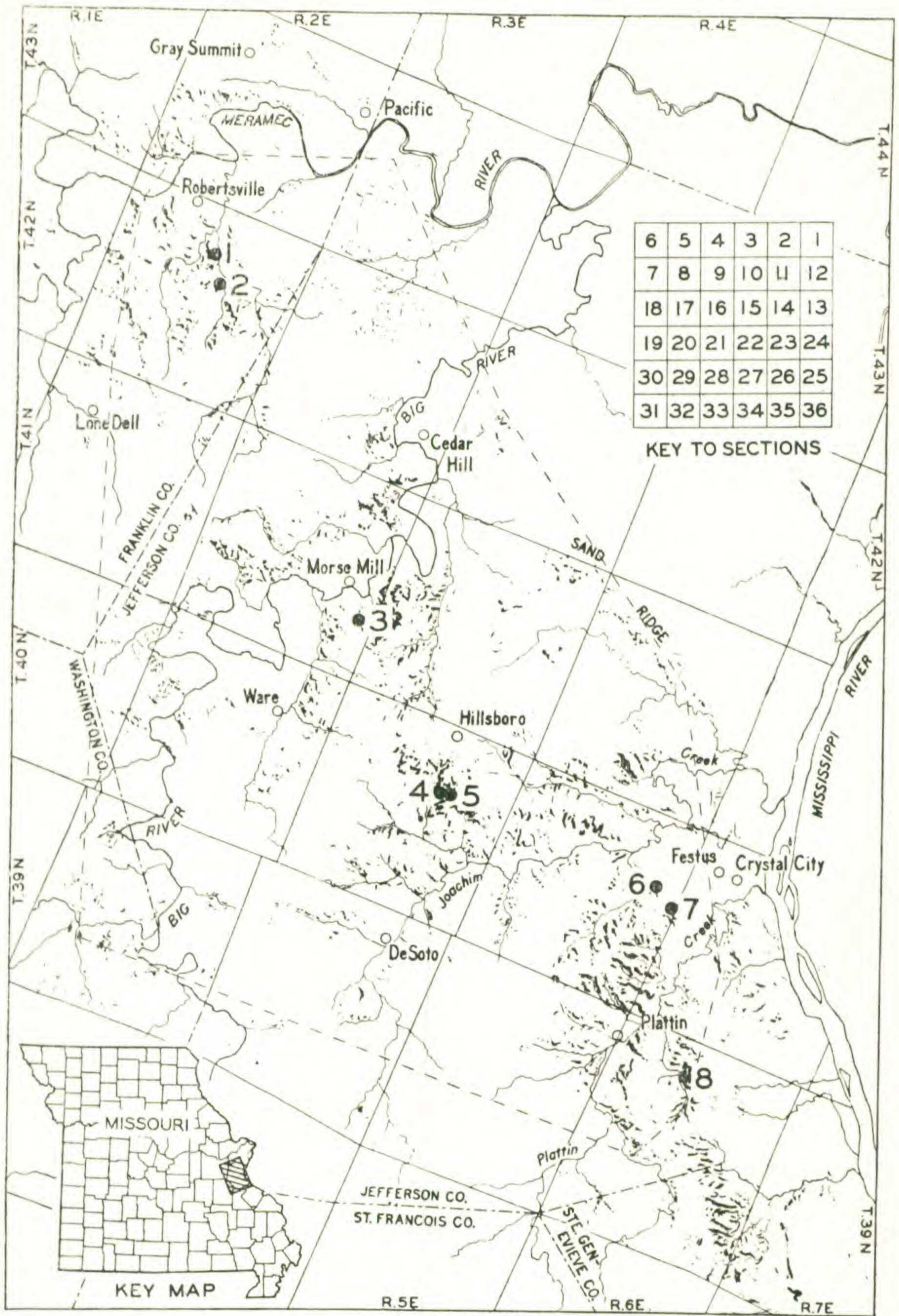


Fig. 2. Glades at which population density studies have been made. Glade numbers correspond with those of Table I.



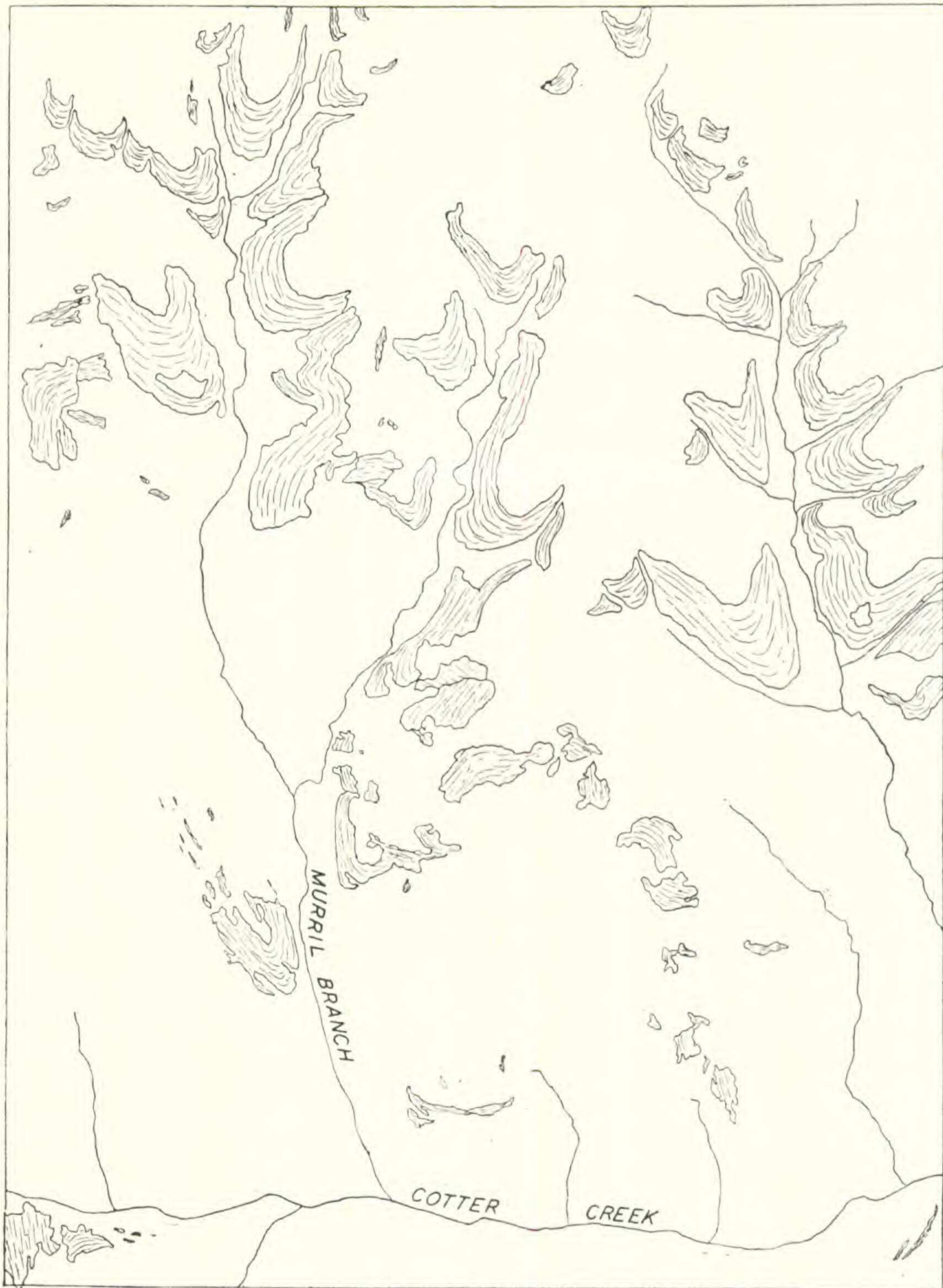


Fig. 3. Two clusters of glades in R.4E, T.40N, S.10, 11, 14, 15, 22 and 23, illustrating their relationship to the drainage pattern. Figure is a reduction of tracings of aerial photographs. Width of figure is two miles.



The next higher category of organization is seen in fig. 2 as a tendency for the entire distribution range to fall into four *regions* of glade concentration: (A) south of Robertsville, (B) about Morse Mill, (C) south of Hillsboro and (D) about Plattin. Scattered glades occur outside these regions of concentration. It is probable that the factors responsible for this large-scale grouping of the glades are variations in thickness of the determining strata of thin-bedded dolomite and the amount of local topographic relief. The four regions appear to be about equivalent in total glade area, but one has the impression from field work that the plant is most abundant on the glades about Plattin, and least abundant in the vicinity of Morse Mill, with the Robertsville and Hillsboro regions intermediate.

TABLE II  
HIERARCHY OF SUBDIVISIONS OF THE *CLEMATIS* POPULATION  
(Compare with fig. 4)

Subdivision	Number	Total area (sq. mi.)	Glade area		Number of <i>Clematis</i>
			(sq. mi.)	(acres)	
Distribution range	1	436	7.0	4,460	1,500,000
Regions	4	100	1.5	980	300,000
Clusters of glades	50	-----	0.09	60	30,000
Colonies (= glades)	1,450	-----	-----	2 (0.1-80)	970 (1-32,000)
Aggregates	15,000	-----	-----	0.2	97

It is thus seen that the distribution of *C. Fremontii* var. *Rieblii* falls naturally into a hierarchy of subdivisions, reminiscent of the hierarchy of subdivisions of the population of *Linanthus Parryae* which Wright ('43) devised for statistical reasons, but differing in that they have a natural basis and show no approach to equality in size. Some speculative calculations can be made of the relative size and number of the subdivisions (Table II). The estimate made in a previous paper (Erickson, '43b) of the total area over which *Clematis* is distributed stands; while several new records could now be added to the map, none are beyond the limits shown there. The calculations made in that paper of the total number of plants have been revised to include all the density data used in compiling Table I. The total was found to be 2,191,000, in gratifying agreement with the previously quoted estimate of 2,200,000 (rounded off from 2,197,000). However this figure has been reduced arbitrarily to 1,500,000, since the density counts weighted large glades too heavily. The estimate of the total number of glades, 1450, has been calculated by assuming that the 60 glades counted in the 18 sq. mi. at R.3E, T.41N, S.1-18, can be considered representative of the entire area. The average number of plants per glade, 970, has been obtained by dividing the number of glades into the total number of plants for the entire area. Similar calcula-



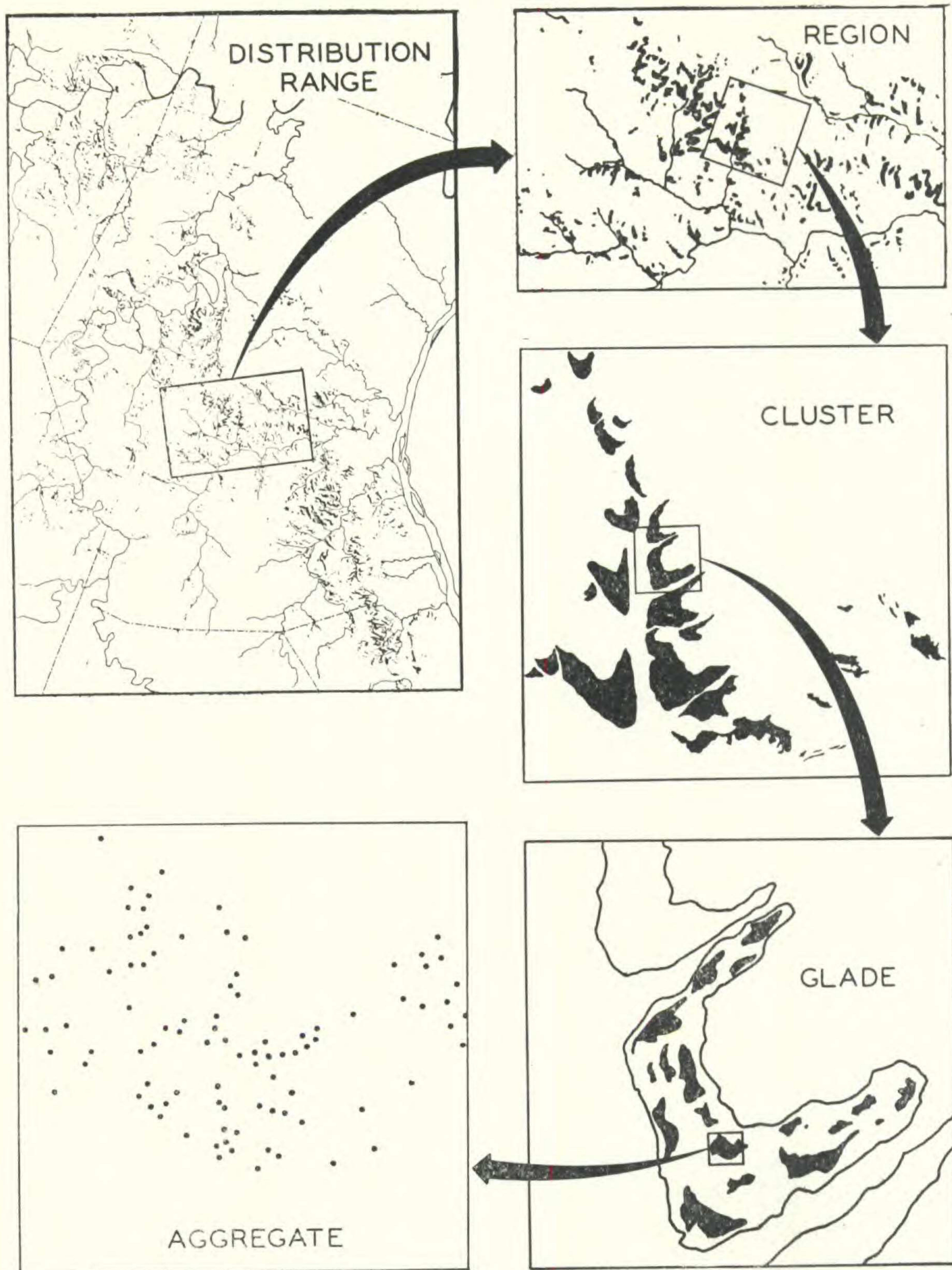


Fig. 4. Diagram to illustrate organization of the distribution range of *C. Fremontii* var. *RiehlII* into a hierarchy of subdivisions: regions of glade concentration; clusters of glades; glades; and aggregates of *Clematis* on glades. Compare with Table II.



tions, with liberal rounding-off of numbers, have given the other values in the table. An indication of the variation in numbers of plants has been given in parentheses for the glades. The other subdivisions also vary greatly in area and number of plants. The organization of the population into a hierarchy of subdivisions is illustrated diagrammatically in fig. 4.

On purely geographical grounds, then, *C. Fremontii* var. *Rieblii* can properly be described as a large population broken up into partially isolated groups. The partially isolated groups of greatest evolutionary significance are probably the aggregates of plants found to occur on each glade, difficult as they are to define in terms of area or number of plants. However, the concept of partial isolation applies equally well to the larger categories, the glades, the clusters of glades, and regions.

#### CONSTANCY OF NUMBERS

Great fluctuations in population size are known to occur in many organisms. Elton ('42, and other publications) has shown this to be the case for many northern mammals, and it is true of some species of *Drosophila* (see, for example, Spencer, '41). *Linanthus Parryae*, an annual plant which has been the subject of a population study, is reported to vary greatly in numbers from year to year (Epling and Dobzhansky, '42). Since the smallest size to which a population may be reduced largely determines its effective size for evolutionary purposes, the possibility of such fluctuations in this *Clematis* population must be considered. Albertson ('42) states that many plants of *C. Fremontii* in Kansas were killed during the years of drought from 1933 to 1939. The late drought, however, was not so severe in the Ozarks, which adjoin the Mississippi embayment, as it was on the prairies. This study was not begun long enough ago to have permitted any first-hand observations, but Anderson ('43) states that the drought of 1936 did not greatly harm many of the glade plants. Its main effect was to check *Andropogon scoparius*, so that other species which are normally held back by competition with it showed an unusually large display of flowers in the immediately following years. No specific observations of *C. Fremontii* var. *Rieblii* were made, but Anderson's opinion is that whatever damage it suffered during the drought was more than balanced by the release of competition from *Andropogon*. It might also be added that the habit of *Rieblii* of completing its growth by the middle of June probably contributes to its ability to withstand drought.

The influence of grazing on the numbers of *Clematis* is manifested in a similar way. The leaves, besides being very leathery when mature, are exceedingly acrid (Greshoff, '09, reports the presence of hydrocyanic acid in *C. Fremontii*), and cattle avoid them. The only evidence of disturbance by livestock is an occasional young shoot which has been nipped off when an inch or so above ground, presumably by error, and flowers which are occasionally removed without disturbance to the leaves. Grazing, however, keeps back the grasses, such as *Andropogon*, and the ultimate effect is to allow *Clematis* to increase both in numbers and in the



size of individual plants. This is strikingly seen in some cases where a fence divides a glade into a grazed and ungrazed portion. The plants on the grazed portion are noticeably larger, and flower somewhat earlier than those on the ungrazed part. Another biotic factor may be mentioned. The plant is subject to sporadic attacks by blister beetles, *Epicauta marginata*, which devour the leaves. Their attacks, however, are merely an annoyance to the investigator. They occur too late in the season, and are not frequent enough, to influence the population size of the plant seriously.

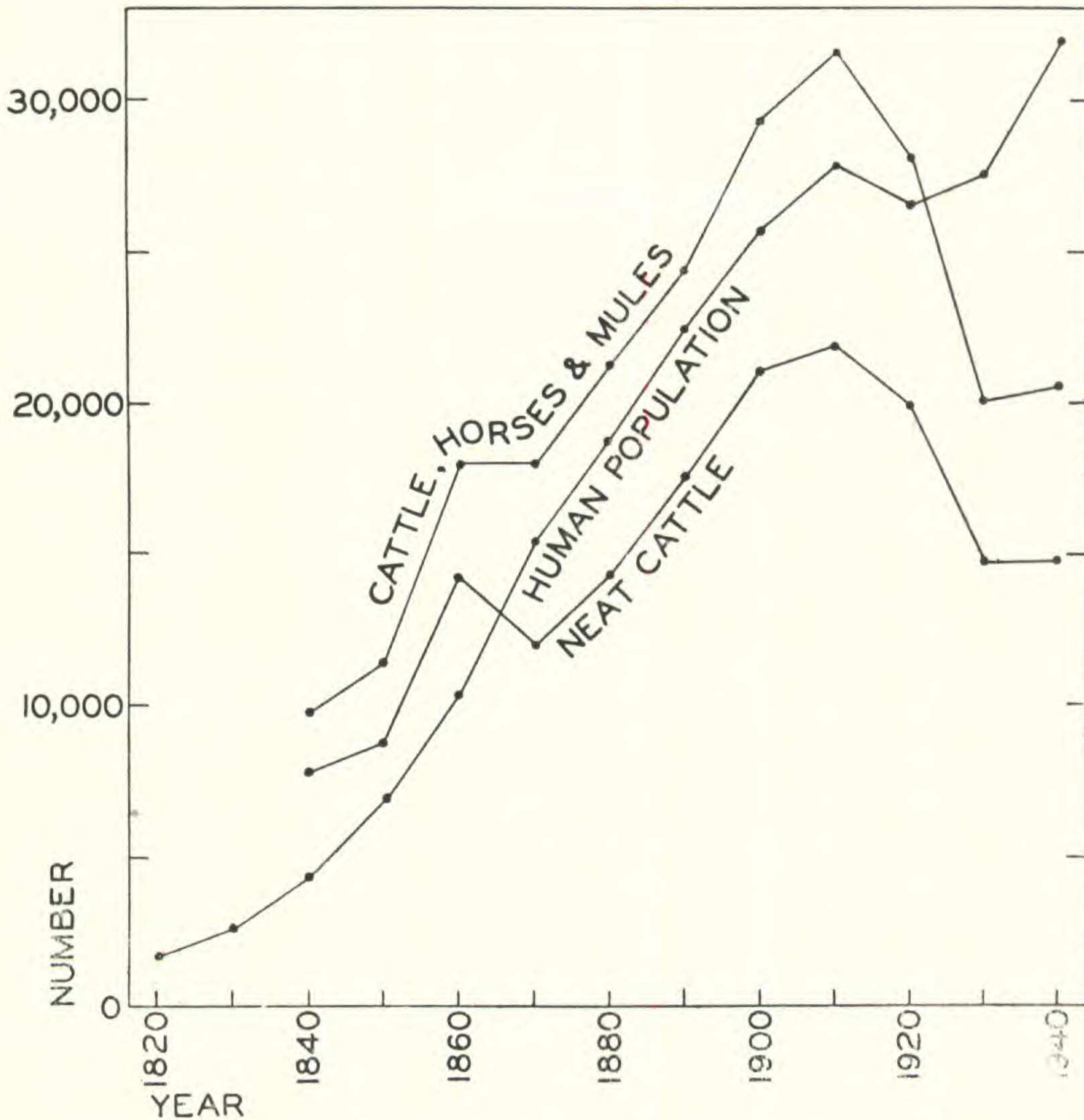


Fig. 5. Data on numbers of livestock and human population in Jefferson Co., Mo., from U. S. Census.

It is presumed that *Clematis* has increased in numbers since the white settlement of the country. United States Census data (fig. 5) show that the livestock population of Jefferson County reached a maximum in 1910, with a considerable decline until a minimum was reached in 1930, since when there has been an increase. Because the numbers of plants is believed to have varied roughly in proportion to the severity of grazing of the glades, it may be concluded that the population size of *Clematis* has increased considerably since 1800, and that it now



has reached relative stability, subject to fluctuations in relation to general economic conditions and changes in the management of individual farms. It is thought that the perennial habit of the plant may serve to damp such influences. Unfortunately, no direct evidence is at hand. A study of the old records of collection of the plant (Erickson, '43b) makes it seem probable that no conspicuous extension or restriction of range has occurred since the 1880's. Certainly no large-scale fluctuation in numbers has occurred in the four years during which the author has observed the plant. On the whole, the size of individual colonies appears remarkably stable as compared with the spectacular fluctuations which are known to occur in some other organisms.

The apparent constancy in size of this *Clematis* population at present does not, of course, imply that there have been no restrictions or extensions of its range in geological time. The presence of the very closely related *C. Fremontii* in Kansas suggests that it and *C. Fremontii* var. *Rieblii* must at one time have had a continuous distribution. A study of the distributions of other glade plants, particularly *Oenothera missouriensis*, suggests that the two *Clematis* populations may have been connected by way of the Edwards Plateau of Texas (unpublished maps prepared by Edgar Anderson). The separation into two populations may have occurred during the semi-arid period of late Pleistocene, or, in view of the importance of competition from grasses, during the warmer, moister period which followed (Sears, '35).

#### LIFE HISTORY

*Clematis Fremontii* var. *Rieblii* is a herbaceous perennial with a woody stem and remarkably coriaceous, prominently veined leaves, which have inspired the common name, "leatherleaf." It flowers during the last week of April and the first week of May, though it was seen flowering sporadically in September, 1941, a month of unusually high rainfall. Growth is completed within three weeks or a month after flowering, and the plants remain green for eight or ten weeks, turning brown during July. Because of their woody nature, many of the stems remain in place until February or March of the following year, the leaves by that time having become skeletonized and weathered to attractive gray laceworks of veins. However, some of the plants have been blown free of their moorings by October. A large plant forms a roughly spherical mass of rigid stems and leaves, and when it is freed, it may be carried for some distance over a glade as a tumbleweed.

The persisting structure is a woody caudex (fig. 6), provided with a mass of brown fleshy roots, in which the reserve food is starch. Two or four lateral buds are formed in the fall at the lower nodes of the old stem, one or more of which unfolds the next spring to form a new shoot. This process, over a period of years, gives rise to a certain amount of branching of the caudex, the older portions of which are torn apart by the growth of the roots. A large plant may consist of perhaps 20 shoots, arising from four or five separate caudices. Branching of the



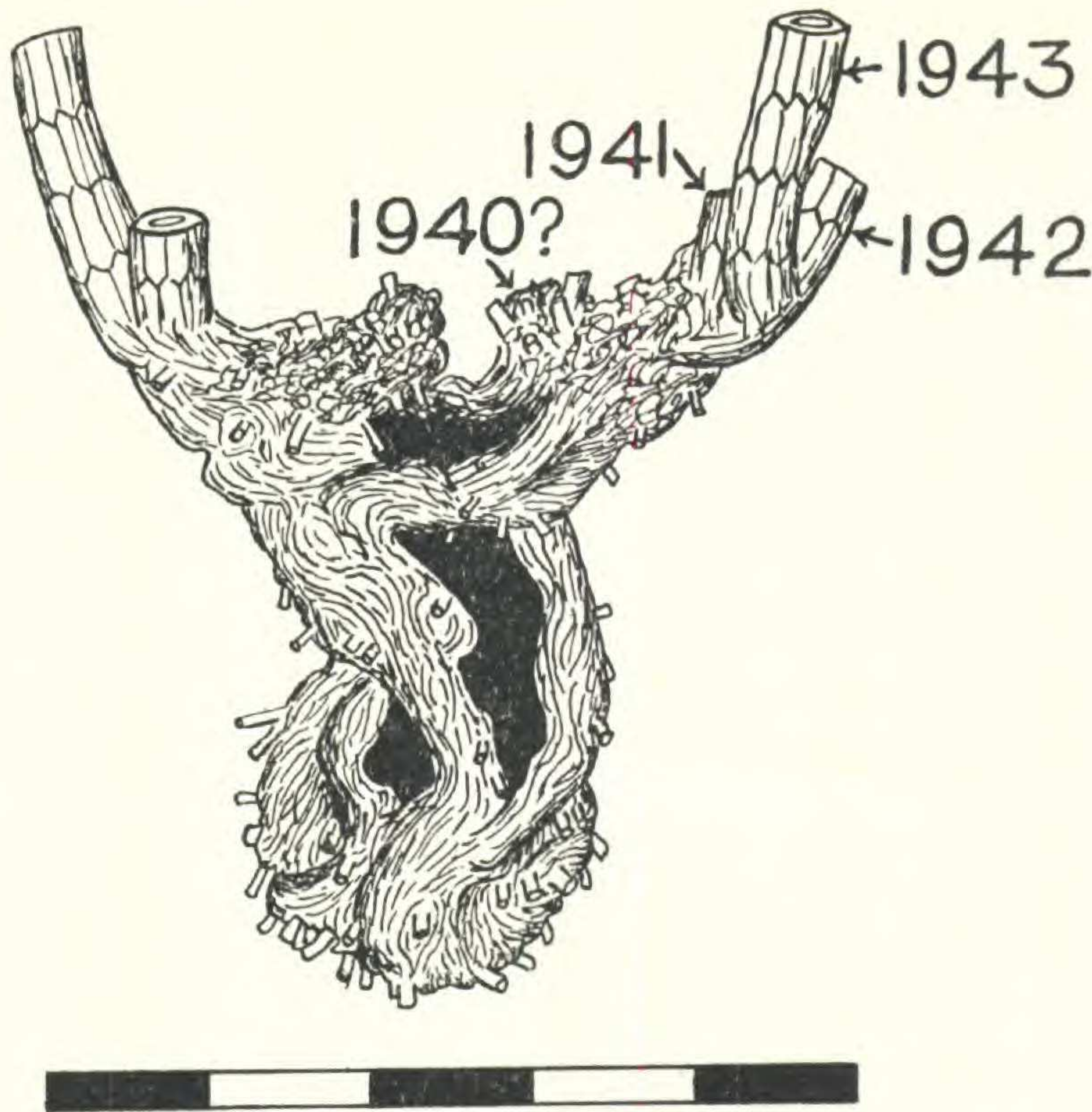


Fig. 6. Caudex of *C. Fremontii* var. *Rieblii*, which supported two shoots in 1943. It was collected in June, and lateral buds had not yet developed. Fleshy roots have been removed. Scale in centimeters.

caudex apparently does not provide a very efficient means of vegetative propagation. Morphological variation from plant to plant is sufficient to permit genetic individuals to be distinguished with some certainty. In a careful examination of perhaps 200 plants for evidences of clonal reproduction, only one case was found in which two separate plants appeared to belong to the same clone. They were about one foot apart, and there was evidence that the separation was accidental, caused by the fall of a tree trunk over the original clump. Three or four clumps were found which were actually two plants. In the population density studies reported above, each plant (or clump) old enough to have flowered has been scored as an individual regardless of its size or number of shoots.

In most species of *Clematis* the achenes are provided with conspicuous plumose tails, presumably well adapted to wind dispersal (fig. 7). In *C. Fremontii* var. *Rieblii*, however, the achene-tails are naked for the greater part of their length, though their basal portions and the apices of the achenes are silky (fig. 8). They are not suited for wind dispersal in the usual sense. Dispersal by the fur of a mammal is hard to visualize, and no evidence has been seen of their use as food by a bird or mammal. Some dissemination is probably achieved by the tumbleweed habit of the largest plants, but most of the achenes merely fall to the base of the parent plant. Dispersal of the achenes over a single glade is probably adequate, but the transportation of achenes from one glade to another must be a rare occurrence.



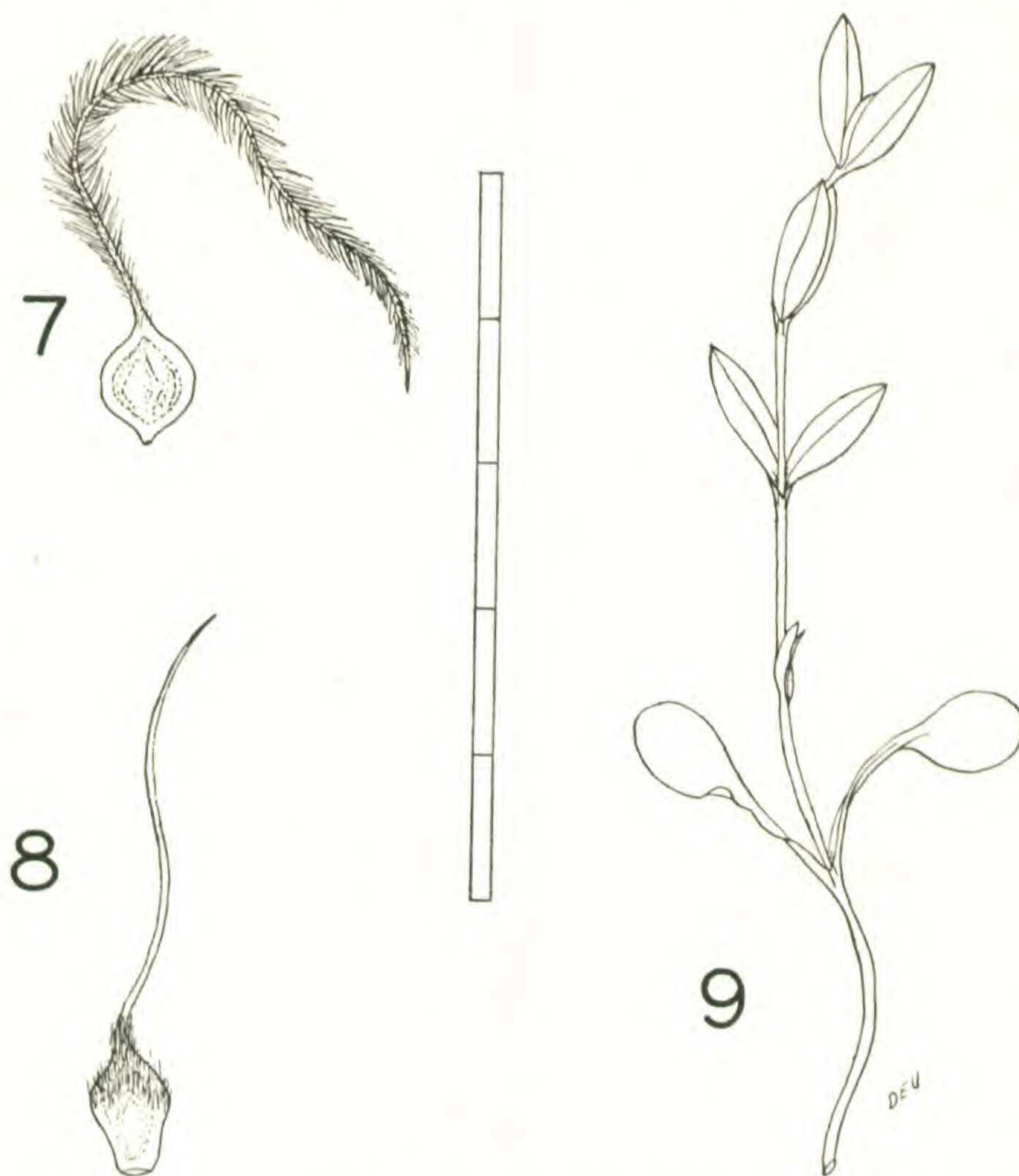


Fig. 7. Achene of *C. Viorna*, showing plumose achene-tail. Fig. 8. Achene of *C. Fremontii* var. *Rieblii*, showing naked achene-tail. Fig. 9. Seedling of *C. Fremontii* var. *Rieblii*, perhaps one month after germination. Scale in centimeters.

Attempts to germinate seeds under greenhouse conditions have been largely unsuccessful. Of about 600 seeds planted, three have germinated. Better success would perhaps be had by layering, but the experiments have not been carried out. Indications are that the percentage of germination of seed in the field is also low. The seedlings escaped detection in the early field work, because of their minute size (fig. 9). Many small sterile plants were found, but examination of the caudex always showed them to be two or more years old. When the seedlings were finally recognized, little trouble was had in finding them on any glade where careful search was made. They are not abundant. Presumably achenes are dropped in the vicinity of every large plant every fall; groups of seedlings can be found in such places in perhaps one case in 100. The conditions required for germination are not well understood, but adequate shade appears to be one of them. Germination takes place in the spring. Seedlings have not been found in September and October, though these months are often characterized by warm rainy weather, similar to that of April.



Growth of the plants seems to be quite slow, four or five years apparently being required from germination until the first flower is produced. Young, sterile plants (fig. 10) have been found on all the glades which have been studied. At R.4E, T.40N, S.15D, 61 of the 528 plants counted, or 8.7 per cent, were such plants. The conclusion that four or five years are required before flowering is based on examination of the caudices of many sterile plants, and of young plants which have produced a single flower. It is more difficult to estimate the age of larger clumps, since the older portions of a caudex are badly fragmented, and annual rings in the wood of the caudex are quite indefinite. Deduction from the size of the plant and of the caudex places the age of large clumps, such as illustrated in fig. 11, at 15 or 20 years, though there is no reason for thinking that they may not be indefinitely older.

#### POLLINATION

Since vegetative reproduction and seed dispersal are quite inefficient, pollen transportation must be looked to as the principal means of gene exchange from one glade to another, and perhaps also from one portion of a glade to another. The flowers are insect-pollinated. They are protogynous, as will be seen from fig. 12, and produce nectar at the base of the stamens. In view of these facts, cross-pollination would seem to be the rule, and more will be said about that below. However, the filaments elongate after the anthers have dehisced, and in an old flower the inner anthers are in contact with the style-tips, so that self-pollination is at least mechanically possible. Glassine bags have been placed over a number of flowers before anthesis to determine the seed-set in enforced self-pollination. The results have been nearly inconclusive. In the first attempts the bags were fastened around the peduncles of flowers, and with one exception failed to stay in place. When the bags were placed over several leaves as well as the flower, the plant and bag were blown over in the wind, became wet, and in most cases molded. Of 106 bags which were placed and later collected, three contained a full head of achenes, and three a few seeds each. The failures to set seed are attributed to the injury done the plant by enclosing it. Normally seeds are set by all the flowers except the smallest ones which occur late in the season on weak branches. Tentatively it is perhaps safe to assume that a plant will be self-pollinated if cross-pollination does not occur first.

*Clematis* is visited by a variety of insects while it is in flower. An insect net was carried for seven days during April, 1943, and as many as possible of the insects found on the flowers were captured. The specimens have been identified by Mr. Harold I. O'Byrne, and Mr. Richard Froeschner, with the exception of some smaller Hymenoptera. The data are presented in Table III. The most frequently found insects are four species of Pentatomidae. They are typically found lurking at the base of a flower, often with the proboscis inserted into one of the fleshy sepals. It is doubtful whether they are concerned in pollination, since they rarely venture to the opening of the flower, and apparently do not move from one plant to another often. The most conspicuous visitors, in order of the



TABLE III  
INSECTS COLLECTED ON *CLEMATIS* FLOWERS

Order Family Species	Number of specimens		
	♂	♀	Total
Homoptera			
Cicadellidae			
<i>Oncometopia lateralis</i> (Fab.)		1	1
Hemiptera			
Pentatomidae			
<i>Euschistus variolarius</i> (Beauv.)	19	3	22
<i>Eu. euschistoides</i> (Voll.)	4	1	5
<i>Thyanta custator</i> (Fab.)	2	2	4
<i>Peribalus limbolarius</i> Stål.		1	1
Neididae			
<i>Neides muticus</i> (Say)	2	2	4
Lepidoptera			
Papilionidae			
<i>Papilio ajax</i> Linn.	1	1	2
<i>P. troilus</i> Linn.		2	2
<i>P. philenor</i> Linn.	1	1	2
Lycaenidae			
<i>Strymon melinus</i> Hbn.	1		1
<i>Everes comyntas</i> (Godt.)	1	1	2
Hesperiidae			
<i>Proteides clarus</i> (Cram.)	2	1	3
<i>Thorybes pylades</i> (Scud.)		1	1
<i>Th. bathyllus</i> (Ab. & Sm.)	3		3
<i>Erynnis brizo</i> (Bdv. & Lec.)	1		1
Sphingidae			
<i>Hemaris diffinis</i> (Bdv.) form <i>tenuis</i> Grote	5	6	11
Coleoptera			
Dermestidae			
<i>Cryptorhophalum picicorne</i> Lec.			1
Melyridae			
<i>Collops vicarius</i> Fall	1		1
Hymenoptera			
Bombidae			
<i>Bombus impatiens</i> Cresson		2	2
<i>B. americanorum</i> (Fab.)		3	3
Apidae			
<i>Apis mellifica</i> Linn.			1
<i>Unidentified</i>			
Hymenoptera (5 species ?)			12
Arachnida, Thomisidae			15

frequency with which they have been seen on the flowers, are the hawk moth, *Hemaris diffinis*, the bumblebees, *Bombus impatiens* and *B. americanorum*, and the swallowtails, *Papilio ajax*, *P. troilus* and *P. philenor*. They alone of the insects captured have proboscides long enough to reach the nectaries from the opening of the flower, a distance of about two cm. It is doubtful whether *Hemaris* or the *Papilio* species are involved in pollination to a considerable extent. The manner in which they cling to the recurved tips of the sepals while obtaining nectar suggests that they may be able to visit many flowers without picking up





Fig. 10. Plant of *C. Fremontii* var. *Rieblii* estimated to be four years old. Scale in centimeters. Fig. 11. Mature plant of *C. Fremontii* var. *Rieblii* probably 15 years old or older. Scale in centimeters. Fig. 12. Flower of *C. Fremontii* var. *Rieblii*. Note that styles are exerted. Anthers have not yet dehisced.



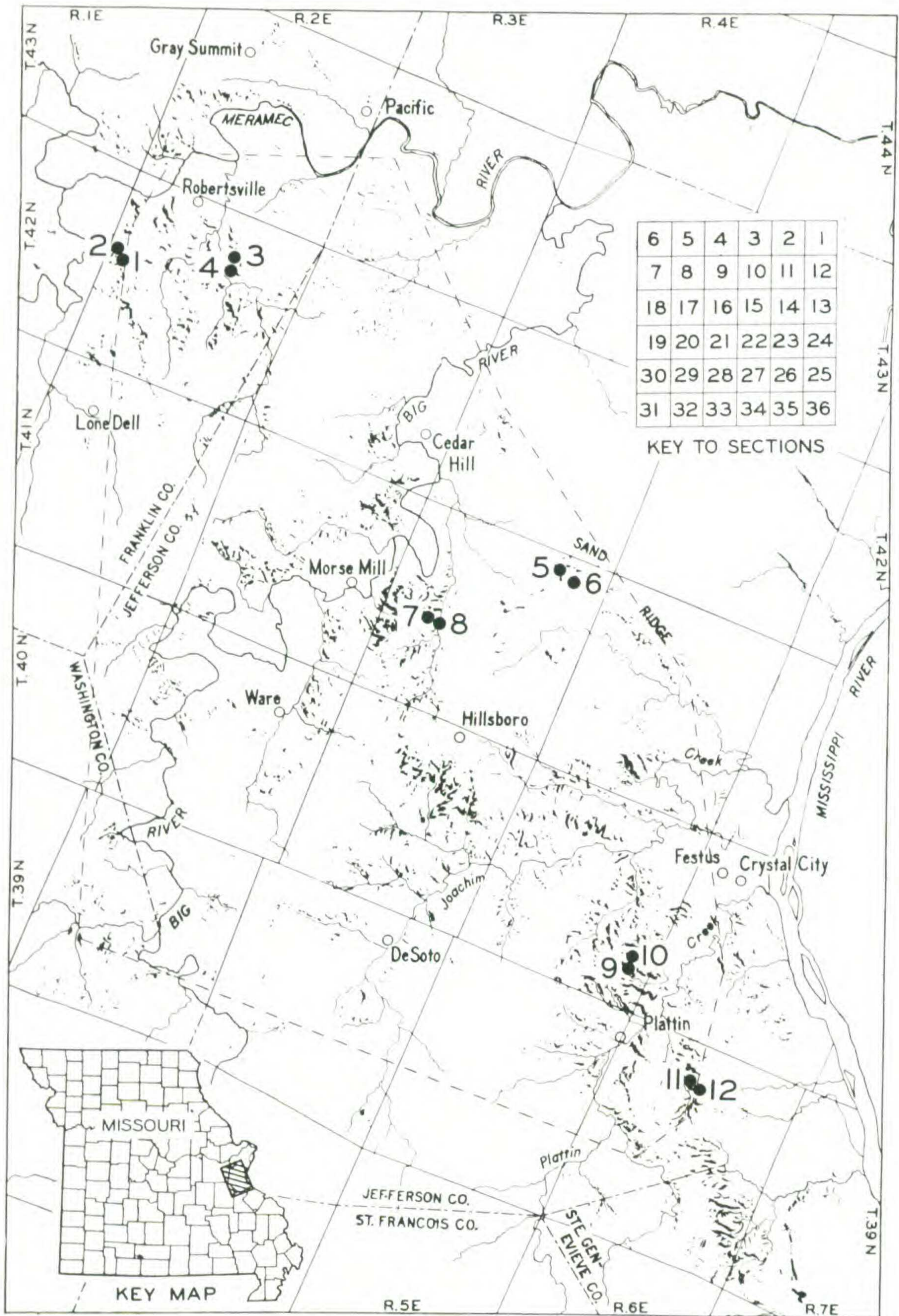


Fig. 13. Glades at which frequency of colored sepal tips has been determined. Glade numbers correspond with those of Table IV.



much pollen. No pollen grains have been detected on the pinned specimens with a hand lens. The bumblebees are undoubtedly queens who have recently come out of hibernation. In late April, they have just begun the establishment of nests (Frison, '27), and are engaged in collecting nectar rather than pollen. This is borne out by the fact that the corbiculae of all the specimens are empty. However, some pollen has been found clinging to the hairs of the head and the prothoracic legs of all of the pinned specimens. It is easy to understand how the bumblebees pick up this pollen. Their behavior at the flowers is much cruder than that of the hawk moths and the swallowtails. Instead of hanging daintily from the sepal tips and probing discretely for nectar, a bumblebee appears to be struggling in an attempt to ram its entire head into the flower as far as possible. The visits of the smaller butterflies of the Lycaenidae and Hesperidae were puzzling at first. It was obvious that they are unable to reach the nectar by the normal route. Closer observation of several individuals showed that they insert their proboscides at the base of the flower, between the valvate margins of two sepals. By this means, of course, they completely avoid contact with the pollen. Old flowers, from which the sepals are about to drop, often swarm with black ants. The ants undoubtedly come in contact with pollen, but it is doubtful whether a single ant visits many flowers in a short period of time, or visits flowers which are young enough to have receptive styles. The honeybee specimen, *Apis mellifica*, and several of the unidentified smaller bees are well loaded down with pollen. They and the bumblebees are certainly the most important pollinators of this *Clematis*. Other species listed in Table III are probably accidental visitors; they could have been collected more efficiently by sweeping. An interesting sidelight on the insect relations of *Clematis* concerns the crab spiders (Thomisidae), of which 15 specimens were obtained. The writer was fascinated on one occasion to watch a *Hemaris* hovering before a flower, and to see it attacked and killed by a spider which had been waiting at the base of the flower.

During the 1942 season some notes were taken on the frequency of insect visits to the flowers. The observations were made without the disturbance caused by attempts to capture the visitors, and were incidental to other work. In an estimated 15 hours on seven different glades, during which an average of perhaps 20 plants were under close enough observation to insure detection of a pollinating insect, nine bumblebees were observed to visit a total of 24 flowers, two honeybees visited one flower each and flew out of sight, one small bee was observed on a single flower, five *Papilios* visited a total of 17 flowers, four *Hemaris* were observed, and one visit by an unidentified smaller butterfly was made. In all of the observations of insects, the writer has been impressed with the great variation from one glade to another. For instance, few hawk moths were recorded in 1942, while in 1943, when other glades were visited, they appeared to be the most frequent visitors, mainly because of the large numbers encountered on a single glade at R.2E, T.42N, S.15B. If any reliance can be placed on the crude estimates made above, it would seem that there is ample provision for the cross-



pollination of a plant within two days of anthesis. Actually, the frequency of pollination is probably higher; the smaller bees were not recorded because their visits failed to attract the writer's attention from other activities and no observations of nocturnal insects were made. Since the bumblebees and the honeybees are reputed to forage over wide areas, the occasional transport of pollen from one glade to another seems quite probable. While working on a single glade, the bumblebees do not systematically go from one flower to its nearest neighbor, but may fly several yards between visits. In a large colony of *Clematis* it seems probable that the circle of possible mates for a given plant may well include a few hundred individuals.

#### PATTERN OF DIFFERENTIATION

Data on the distribution of gene frequencies within a population provide the most useful information for evaluating the relative roles of selection and random differentiation. However, the collection of such data presupposes a basic fund of knowledge of the genetics of an organism which exists in relatively few cases. Lacking that for *Clematis*, a careful examination of many plants has been made for a morphological character which can at least be scored as present or absent, in the hope that eventually it might turn out to have a simple genetic basis. There is considerable variation in flower color, the outer surfaces of the sepals ranging from the blue and purple of the manuals, to practically white. Most flowers in anthesis are nearly white, with considerable variation in the distribution of the small amount of color which is present. It is suspected that true albino flowers exist, but they cannot be distinguished with certainty from those in which the pigment is very dilute.

The inner (adaxial) surfaces of the recurved sepal tips, however, show a discrete variation in color which is suggestive of a simple mode of inheritance, and a number of plants have been scored for presence or absence of color (pink or blue) at this place. A collection of 36 or fewer flowers was made on each of 12 glades, so selected that they could be arranged in pairs. The two glades of a pair are on adjacent ridges (fig. 13) about 0.35 mi., or 1850 ft., apart on the average. Two pairs of collections, 4.1 mi. apart on the average, were made in each of three regions. Glades 1-4 in the Robertsville region are about 28.5 mi. from glades 9-12 in the Plattin region, and glades 5-8, in the Morse Mill region, are midway between. The number and proportion of flowers with colored sepal tips in each collection, in each pair of collections, and in each region are shown in Table IV. The proportions for the three regions, 0.36, 0.11, and 0.05, suggest a "cline" (Huxley, '38), the frequency of colored sepal tips being greatest in the Robertsville region and decreasing toward the southeast.

In examining the data statistically, the assumption that the population is really uniform in proportion of colored sepal tips has first been tested by the  $\chi^2$  test. Theoretical frequencies of colored sepal tips have been calculated by multiplying the total number of flowers in each collection by the over-all proportion,



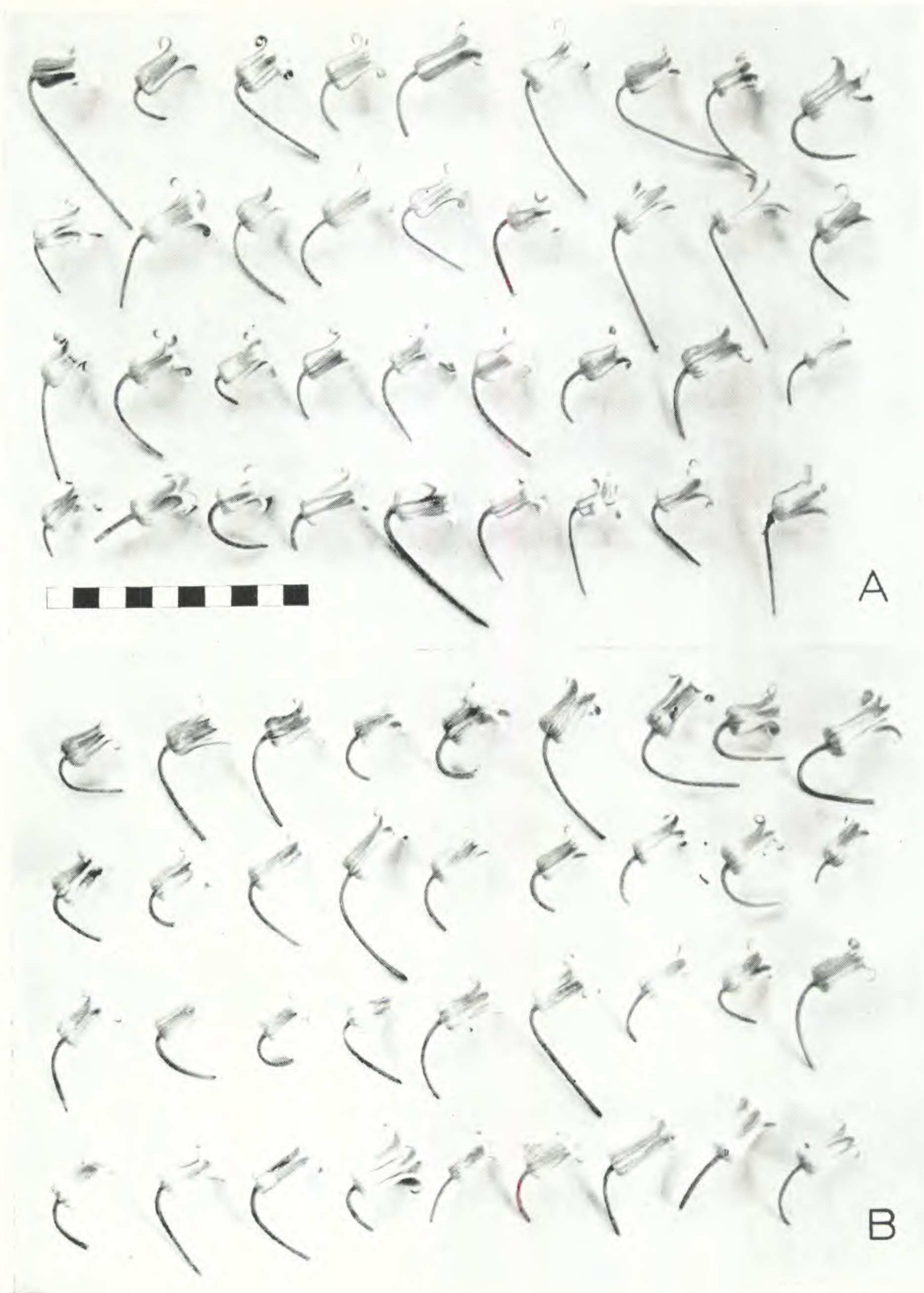


Fig. 14. Two collections of flowers of *C. Fremontii* var. *RiehlII*, obtained at R.2E, T.42N, S.18H (A), and at R.6E, T.39N, S.4P (B), to illustrate nature of morphological variation. Scale in centimeters.



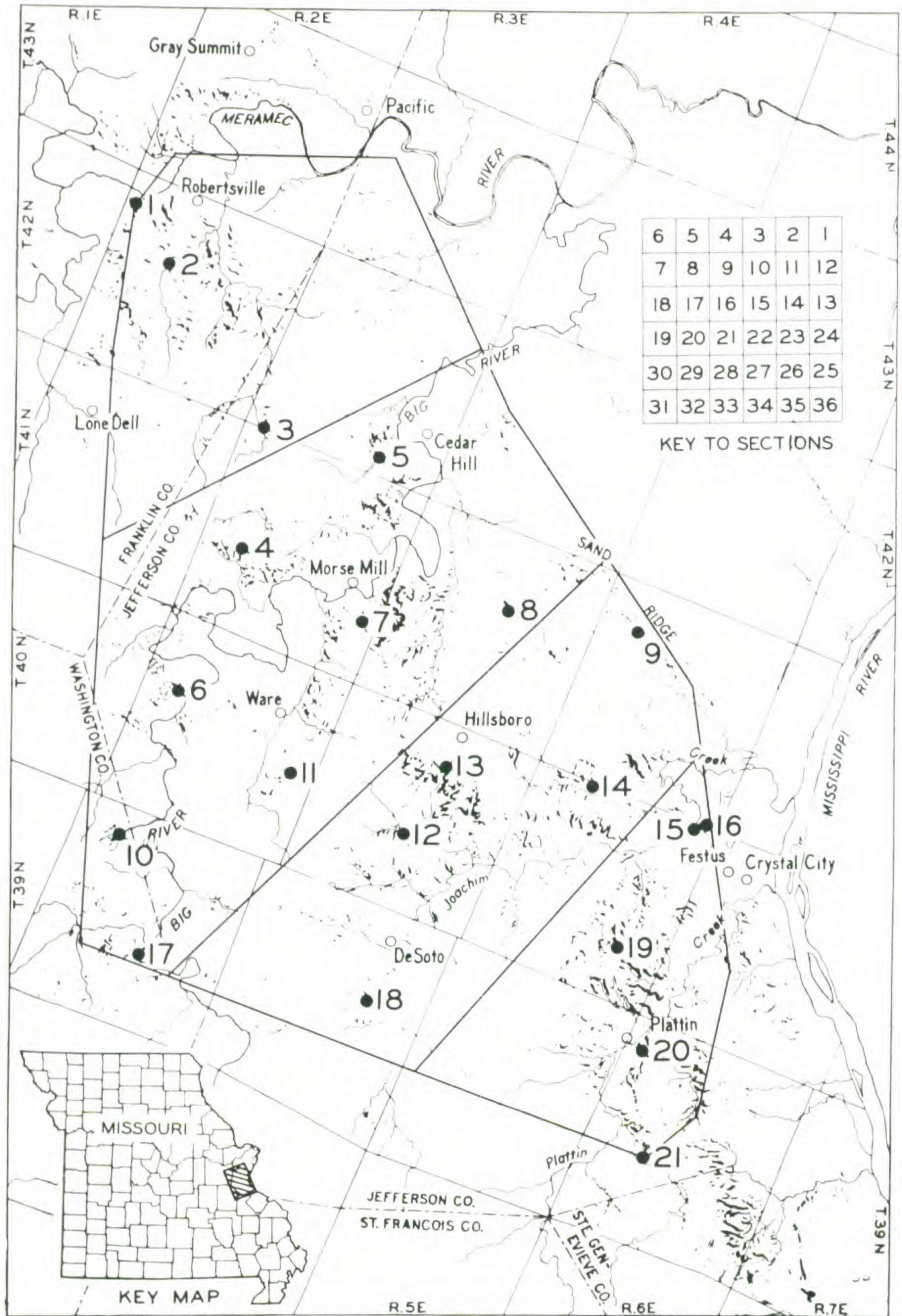


Fig. 15. Glades at which measurements have been made of sepal length, sepal width, margin width, and sepal coil. Glade numbers correspond with those of Table V.



TABLE IV  
 FREQUENCY OF COLORED SEPAL TIPS  
 (Glade numbers correspond with those of fig. 13)

No.	Location	Number of flowers			Number and proportion with colored tips			Significance of difference between proportions			
		Glade	Pair	Region	Glade	Pair	Region	Betw. glades		Betw. pairs	
								x	2P	x	2P
1	R. 2E, T. 42N, S. 18D	31	67	139	11-0.36	26-0.39	50-0.36	0.52	0.61	0.67	0.50
2	R. 2E, T. 42N, S. 18H	36			15-0.42						
3	R. 2E, T. 42N, S. 10E	36	11-0.31		24-0.33	0.52		0.62			
4	R. 2E, T. 42N, S. 10K	36	13-0.36								
5	R. 4E, T. 41N, S. 2E, F	18	54	93	3-0.17	7-0.13	10-0.11	0.57	0.58	0.81	0.42
6	R. 4E, T. 41N, S. 2G	36			4-0.11						
7	R. 4E, T. 41N, S. 20D	14	39		2-0.14	3-0.08		1.16	0.25		
8	R. 4E, T. 41N, S. 20E	25			1-0.04						
9	R. 5E, T. 40N, S. 25F	36	72	144	2-0.06	3-0.04	7-0.05	0.59	0.56	0.39	0.70
10	R. 5E, T. 40N, S. 25B	36			1-0.03						
11	R. 6E, T. 39N, S. 4P	36	72		3-0.08	4-0.06		1.03	0.30		
12	R. 6E, T. 39N, S. 4S	36			1-0.03						

Significance of difference between proportions, between regions:

Betw. glades 1-4 and 5-8:  $x = 4.30$ ;  $2P = 1.6 \times 10^{-5}$

Betw. glades 1-4 and 9-12:  $x = 6.52$ ;  $2P = 1.1 \times 10^{-10}$

Betw. glades 5-8 and 9-12:  $x = 1.72$ ;  $2P = 8.6 \times 10^{-2}$

Betw. glades 1-4 and 5-12:  $x = 7.05$ ;  $2P = 2.0 \times 10^{-12}$

0.178. Carrying through the calculation gives a  $\chi^2$  value of 43.9, with 8 degrees of freedom. The probability for a higher  $\chi^2$  value is less than 0.001, which rules out the possibility that the population is uniform in this character. (In this statistical analysis, and the succeeding ones, the methods and orthography of Rider ('39) have been followed except in the analysis of covariance on p. 446 *et seq.*)

For a more detailed analysis of the data, calculations have been made of the significance of the difference between the proportions for each pair of glades, for the two pairs of glades in each region, and for the three regions. There is no significant difference in proportion of colored sepal tips between any two adjacent glades (Table IV, third column from right), nor between the two pairs of glades in each region (Table IV, last column). The difference in proportion between the Robertsville and Morse Mill regions, between Robertsville and Plattin, and between



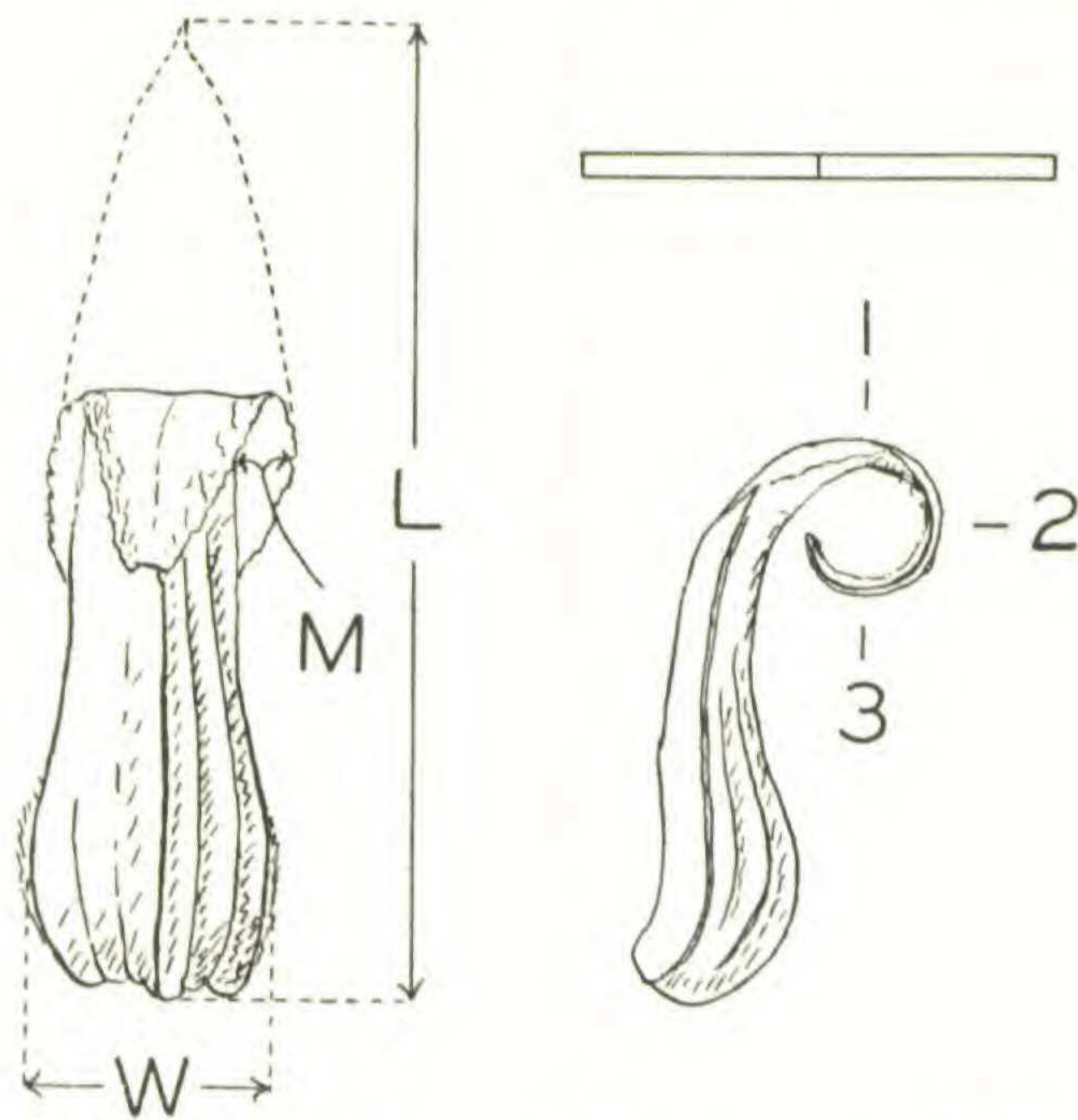


Fig. 16. Sepal of *C. Fremontii* var. *Rieblii*. Drawing at left illustrates manner in which measurements of sepal length, L, sepal width, W, and margin width, M, were made. Drawing at right illustrates method of scoring "sepal coil" in quadrants. Scale in centimeters.

the Robertsville region and the remaining glades, however, is highly significant (Table IV, bottom). Whether there is a real difference between the Morse Mill and Plattin regions is doubtful. The northwest-southeast differentiation in this character may be described as a cline, but its most significant feature is the deviation of the plants of the Robertsville region.

The flowers were brought to one place so that they could be studied at one time and photographed. Examination of the flowers indicated that the differentiation in proportion of colored sepal tips is correlated with similar differentiation in the color of the entire sepal. Perhaps it is merely an expression of the latter.

The flowers also show evident differences in a number of continuously varying characters, as illustrated in fig. 14. Measurements of four such characters have been made on another series of glades. The 21 glades at which measurements, usually of 35 flowers, were made are indicated in fig. 15. They are scattered throughout the distribution range. Measurements of the distance between each of the 210 pairs of glades have been made with dividers on a map (scale:  $\frac{1}{4}$  in. = 1 mi.), averaged, and the average converted to miles. This yields an average distance between the glades of 12.8 mi.

In making the flower measurements, care was taken to select flowers only from the primary shoots of mature clumps. The flowers which terminate the primary shoots of a single clone are remarkably similar in size, coloration, and general aspect. Those which terminate secondary branches are often smaller, later in anthesis, and darker in color. None of the latter have been included in the measurements. The sampling scheme has been to select flowers from a re-



TABLE V  
MEASUREMENTS OF FLOWERS  
Means and Standard Deviations for Glades  
(Glade numbers correspond with those of fig. 15)

No.	Location	N	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil quadrants	
			$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$
1	R. 2E, T. 42N, S. 7A	45	33.56	2.93	8.84	0.54	2.03	0.47	3.29	1.05
2	R. 2E, T. 42N, S. 17B	37	34.08	3.86	9.51	1.04	2.46	0.57	3.41	1.12
3	R. 3E, T. 42N, S. 31D	35	37.54	4.92	10.00	0.98	1.87	0.70	3.06	0.88
4	R. 3E, T. 41N, S. 20A	35	34.20	3.95	9.31	0.93	1.74	0.51	3.46	0.98
5	R. 3E, T. 42N, S. 35B	35	33.09	4.10	9.31	1.07	1.70	0.43	3.31	0.80
6	R. 3E, T. 40N, S. 17A	35	34.20	3.47	10.11	0.97	1.73	0.41	2.74	0.79
7	R. 3E, T. 41N, S. 25D	35	34.66	2.92	9.83	1.19	1.89	0.42	3.69	0.55
8	R. 4E, T. 41N, S. 15B	35	33.14	3.38	10.11	1.15	2.00	0.42	3.20	0.60
9	R. 5E, T. 41N, S. 8B	32	29.72	5.92	9.06	1.08	1.69	0.47	3.34	0.84
10	R. 3E, T. 39N, S. 5D	35	32.91	4.02	9.54	1.39	1.66	0.60	2.97	0.79
11	R. 3E, T. 40N, S. 24B	35	34.46	3.72	9.34	1.03	2.06	0.64	2.63	0.87
12	R. 4E, T. 40N, S. 22C	35	35.06	3.10	10.14	1.28	1.76	0.43	3.11	0.84
13	R. 4E, T. 40N, S. 10E	35	34.37	4.06	9.80	0.97	1.70	0.41	2.80	0.69
14	R. 5E, T. 40N, S. 5D	35	33.80	3.34	9.54	1.19	1.67	0.49	2.77	0.87
15	R. 5E, T. 40N, S. 1B	35	32.37	2.74	9.37	1.28	1.89	0.41	2.89	0.95
16	R. 5E, T. 40N, S. 1C	35	32.66	3.73	9.49	1.09	1.90	0.53	2.71	0.90
17	R. 3E, T. 39N, S. 22B	35	34.60	3.59	10.26	1.21	2.14	0.55	3.00	1.00
18	R. 4E, T. 39N, S. 14A	35	36.57	3.40	10.23	1.48	2.09	0.55	3.17	0.96
19	R. 5E, T. 40N, S. 26B	35	33.89	7.02	9.69	1.96	1.74	0.53	2.71	1.17
20	R. 6E, T. 39N, S. 6J	35	35.97	4.00	9.49	4.09	2.01	0.44	3.14	0.99
21	R. 6E, T. 39N, S. 20B	35	33.34	2.89	9.31	1.12	1.64	0.43	2.97	0.83
Total		744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

stricted portion of each glade, rather than to sample the entire population of the glade. Usually the measurements were begun at a point where the plants were abundant, and a roughly spiral course was followed, during which a flower from each mature plant encountered was measured. No records were kept of the location on the glade of the plants selected. A sepal was removed from each flower, and the measurements indicated in fig. 16 were made with a celluloid rule. The length of the sepal was measured to the nearest mm., after straightening the recurved tip, but no attempt was made to flatten the thick base. Width was



TABLE VI  
MEASUREMENTS OF FLOWERS  
A. Analysis of Variance for Glades

CHARACTER	Sum of squares of deviations	Degrees of freedom	Mean square deviation	<i>w</i>	<i>P</i>
SEPAL LENGTH					
Within glades	11,271.14	723	15.59	5.51	< 0.0001
Among glades	1,717.64	20	85.88		
Total	12,988.78	743			
SEPAL WIDTH					
Within glades	1,158.92	723	1.603	3.60	< 0.0001
Among glades	115.46	20	5.773		
Total	1,274.38	743			
MARGIN WIDTH					
Within glades	202.65	723	0.2803	5.52	< 0.0001
Among glades	30.96	20	1.5480		
Total	233.61	743			
SEPAL COIL					
Within glades	658.84	723	0.9113	3.16	< 0.0001
Among glades	57.66	20	2.8833		
Total	716.50	743			

B. Analysis of Variance for Regions

SEPAL LENGTH					
Within regions	12,906.32	740	17.44	1.58	0.20
Among regions	82.46	3	27.49		
Total	12,988.78	743			
SEPAL WIDTH					
Within regions	1,257.78	740	1.700	3.26	0.020
Among regions	16.60	3	5.533		
Total	1,274.38	743			
MARGIN WIDTH					
Within regions	224.85	740	0.3039	9.61	< 0.0001
Among regions	8.76	3	2.9200		
Total	233.61	743			
SEPAL COIL					
Within regions	705.44	740	0.9533	3.87	0.0089
Among regions	11.07	3	3.6887		
Total	716.50	743			

measured, to the nearest mm., at the widest point, quite near the base of the sepal, without any attempt to flatten it. The width of the expanded sepal margin was measured to the nearest 0.5 mm., at its widest point, usually quite near the tip of the sepal. The degree to which the tip of the sepal is recurved, "sepal coil," was scored by noting the number of quadrants through which the tip has moved in anthesis. Thus, if the sepal tip has turned through 360°, as has the one illustrated, it is scored as 4. The mean and standard deviation of each series of measurements are given in Table V. Because of the relative coarseness of the scale used for three of the measurements (width, margin and coil), Sheppard's correction has been applied in calculating the standard deviations.

Inspection of the table discloses differences in means from one glade to another in each of the characters. In order to determine whether the variation in these characters from one glade to another is greater than that on a single glade (in



TABLE VII  
MEASUREMENTS OF FLOWERS

## A. Means and Standard Deviations for Regions

Region	N	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
		$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$	$\bar{X}$	$\sigma^*$
Robertsville. Glades 1, 2, 3	117	34.90	4.41	9.40	1.24	2.12	0.62	3.26	1.03
Morse Mill. Glades 4, 5, 6, 7, 8, 10, 11, 17	280	33.91	3.72	9.73	1.19	1.86	0.53	3.13	0.88
Hillsboro. Glades 9, 12, 13, 14, 18	172	33.98	4.18	9.77	1.29	1.78	0.50	3.03	0.87
Plattin. Glades 15, 16, 19, 20, 21	175	33.65	4.57	9.47	1.38	1.84	0.49	2.89	0.99
Total	744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

## B. Tests of Significance of Differences between Means for Regions

Between Regions	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
	<i>t</i>	2 <i>P</i>	<i>t</i>	2 <i>P</i>	<i>t</i>	2 <i>P</i>	<i>t</i>	2 <i>P</i>
Robertsville and Morse Mill	2.28	0.023	-2.46	0.015	4.15	< 0.0001	1.12	0.26
Morse Mill and Hillsboro	-0.18	0.86	-0.33	0.74	1.64	0.10	1.06	0.29
Hillsboro and Plattin	0.70	0.48	2.08	0.038	-1.04	0.30	1.49	0.14
Robertsville and Hillsboro	1.79	0.075	-2.40	0.017	5.09	< 0.0001	1.65	0.10
Morse Mill and Plattin	0.67	0.50	2.13	0.033	0.55	0.58	2.68	0.0077
Robertsville and Plattin	2.32	0.021	-0.42	0.67	4.32	< 0.0001	2.64	0.0088

other words, whether these samples must be regarded as representing a number of separate populations or as portions of a single statistical population), the data have been subjected to an analysis of variance. The results are presented in Table VI, A. In each of the four flower characters the probability that the 21 series of measurements can be regarded as portions of the same statistical population is very low, clearly beyond the threshold of significance. This excess of variance among glades over that within individual glades is evidence of considerable local differentiation. It may be ascribed to the partial isolation of the glades, which has been discussed above.

It is also of interest to inquire whether these data demonstrate a cline, or regional differentiation of any sort, in any of the characters. In view of the local differentiation, it has not been possible to find evidence of differentiation on a regional scale by examining the means of individual glades, or by studying a series of ideograms, such as Anderson ('36) prepared from his data on *Iris*. The



sampling scheme used in selecting these glades leaves a good deal to be desired when it comes to investigating the question of regional differentiation. Nevertheless, the data for the 21 glades have been combined into four groups as shown in fig. 15 and Table VII, A. The four groups correspond approximately with the Robertsville, Morse Mill, Hillsboro and Plattin regions, described above. An analysis of variance has been carried out for each of the four sepal measurements (Table VI, B). It indicates that the excess of variance from one region to another over that within regions is significant for margin width and sepal coil, perhaps so for sepal width, and not for sepal length. In other words, there appears to be significant regional differentiation in two (or three) of the four measurements.

It is then worth while to compare the means for each of the regions. Means and standard deviations for each of the measurements have been entered in Table VII, A. The  $t$  value for the difference between each pair of means has also been determined. In this calculation the variance of the difference has been estimated separately for each pair of means. The probability corresponding to each  $t$  value has been found from a table of "Student's" distribution (Table VII, B). In sepal length, the plants from the Robertsville region are perhaps significantly higher than those of the other three regions, while there are no significant differences among the latter. The Robertsville, and perhaps the Plattin, plants have significantly narrower sepals than do those of Morse Mill and Hillsboro. The valvate margin of the sepal is wider in the Robertsville region than in the other three regions, and this difference is highly significant. As in sepal length, the differences in margin width among the Morse Mill, Hillsboro and Plattin regions are not significant. The sepals are most strongly recurved in the Robertsville region and least so around Plattin. The differences in this character between adjacent regions are on the border-line of significance, but the differentiation becomes significant from one end of the range to the other.

In summary, there is significant regional differentiation in each of the four flower measurements. In only one case, sepal coil, can the differentiation be described as a cline, in the sense of a consistent geographical trend. The most striking feature of the differentiation in these characters is the difference between the Robertsville plants and those of the other portions of the population. The same conclusion was drawn above from the analysis of the data on proportion of colored sepal tips (Table IV).

It has been shown above that there is greater differentiation in the flower measurements from one glade to another than on single glades. It may also be inquired whether there is local differentiation from one portion to another of a single glade. To answer this question it is necessary to obtain data on the location on a glade of the plants studied. Such data were not obtained for the plants whose flowers were measured. Laying out quadrats such as those used in population density studies is time-consuming, and it would not have been feasible during the flowering period of *Clematis* to have obtained both flower measurements and accurate locality data for any large number of plants.



On several glades, however, a leaf was collected from each plant plotted during the population density study. The leaves on a single plant vary in size and shape, though those at corresponding positions on different shoots of a single clone are closely similar. To obtain leaves from different plants which would be comparable, one leaf of the pair which subtends the first flower of the plant, or of the most vigorous shoot of a clone, was taken. The leaves of this pair are usually the longest, and comparatively, the widest ones on a shoot. On young sterile plants, however, the apical pair of leaves is usually small, and from such plants a leaf of the largest pair was taken, which was usually at the third or fourth node from the apex. The quadrat in which each leaf was collected was noted on the leaf with wax pencil, or on a small label attached to the leaf with Cellophane tape. Since the leaves are leathery in texture, it was not felt necessary to press them.

Two of the leaf collections have been subjected to measurement and statistical analysis. One of these was obtained over a continuous portion of the large glade at R.6E, T.39N, S.4-O (Glade no. 8 in fig. 2 and Table I). The entire area was laid out in 10-ft. quadrats (fig. 1, B), and a leaf was removed from each plant. Somewhat more than 1,000 leaves were collected. In some cases the label indicating the quadrat in which the leaf was obtained was lost, so that 983 leaves were available for study. Measurements of the dry leaves were made to the nearest mm. with a celluloid rule, some time after collection. Length was measured on the adaxial surface, from the tip to the point of attachment to the stem. Width was measured at the widest place, usually in the proximal half of the leaf. Many of the leaves are not plane, the adaxial surfaces sometimes being markedly concave, and rarely saddle-shaped. In all cases the rule was bent to follow the curvature of the leaf surface. (The author is indebted to John R. Melin, A. S., a U. S. Navy V-12 student, for the measurements, and for assistance in the calculation.)

It may first be inquired whether there are significant differences in the absolute length and width measurements from one portion of the area studied to another. This may be answered, as for the flower measurements discussed above, by carrying out an analysis of variance. For the purpose of the analysis, the area has been subdivided in three ways. The 10-ft. quadrats have first been combined by fours into a total of eighty-eight 20-ft. quadrats, which include an average of 11.17 measured leaves each. The number of leaves in each 20-ft. quadrat varies from 2 to 27 in a non-Poisson manner (see p. 417). Secondly, the 20-ft. quadrats have been combined by fours, with slight irregularities, into a total of twenty-four 40-ft. quadrats, which include an average of 40.96 leaves each, ranging from 9 to 80. The arrangement of the 40-ft. quadrats, and the numbers which have been assigned to them are shown in fig. 1, B. Finally, the entire area has been divided into three strips 80 ft. wide. They consist of the 40-ft. quadrats numbered 1-8, 9-18 and 19-24, and include 435, 435, and 113 leaves respectively. These strips will be referred to as 80-ft. quadrats.

For each of the three schemes of subdivision, calculations have been made of: the sums of squares of the deviations of the length and width of each leaf from



TABLE VIII  
MEASUREMENTS OF LEAVES AT R.6E, T.39N, S.4-0  
Analysis of Variance and Covariance

## A

	$\Sigma x^2$	$\Sigma xy$	$\Sigma y^2$	Degrees of freedom
Within 20-ft. quadrats	249,046.89	186,367.06	199,118.64	895
Among 20-ft. quadrats	77,572.11	71,742.80	76,685.39	87
	For variance in length, $n_1 = 87$ , $w = 3.20$ , $P < 0.0001$			
	For variance in width, $n_1 = 87$ , $w = 3.96$ , $P < 0.0001$			
Within 40-ft. quadrats	279,040.46	210,952.56	226,036.68	959
Among 40-ft. quadrats	47,578.54	47,157.30	49,767.36	23
	For variance in length, $n_1 = 23$ , $w = 7.11$ , $P < 0.0001$			
	For variance in width, $n_1 = 23$ , $w = 9.18$ , $P < 0.0001$			
Within 80-ft. quadrats	314,033.96	244,203.24	260,436.61	980
Among 80-ft. quadrats	12,585.04	13,906.62	15,367.42	2
Total	326,619.00	258,109.86	275,804.03	982
	For variance in length, $n_1 = 2$ , $w = 19.64$ , $P < 0.0001$			
	For variance in width, $n_1 = 2$ , $w = 28.91$ , $P < 0.0001$			

## B

	Sum of squares of deviations	Degrees of freedom	Mean square deviation	$w$	$P$
Within 20-ft. quadrats:					
Average regression	139,462.41	1	139,462.41		
Regression differences	6,060.80	87	69.66	1.05	0.28
Residuals	53,595.43	807	66.41		
	(199,118.64)	(895)			
Among 20-ft. quadrats:					
Regression of means	66,351.55	1	66,351.55		
Residuals	10,333.84	86	120.16	1.81	$< 0.0001$
	(76,685.39)	(87)			
Total	275,804.03	982			
Within 40-ft. quadrats:					
Average regression	179,478.60	1	179,478.60		
Regression differences	2,419.41	23	105.19	1.53	0.050
Residuals	64,138.67	935	68.60		
	(226,036.68)	(959)			
Among 40-ft. quadrats:					
Regression of means	46,739.87	1	46,739.87		
Residuals	3,027.48	22	137.61	2.01	0.0034
	(49,767.35)	(23)			
Total	275,804.03	982			
Within 80-ft. quadrats:					
Average regression	189,900.55	1	189,900.55		
Regression differences	1,075.38	2	537.69	7.56	0.0005
Residuals	69,460.68	977	71.10		
	(260,436.61)	(980)			
Among 80-ft. quadrats:					
Regression of means	15,366.98	1	15,366.98		
Residuals	0.44	1	0.44	161.8	0.062
	(15,367.42)	(2)		$(n_1 = 977)$	
Total	275,804.03	982			



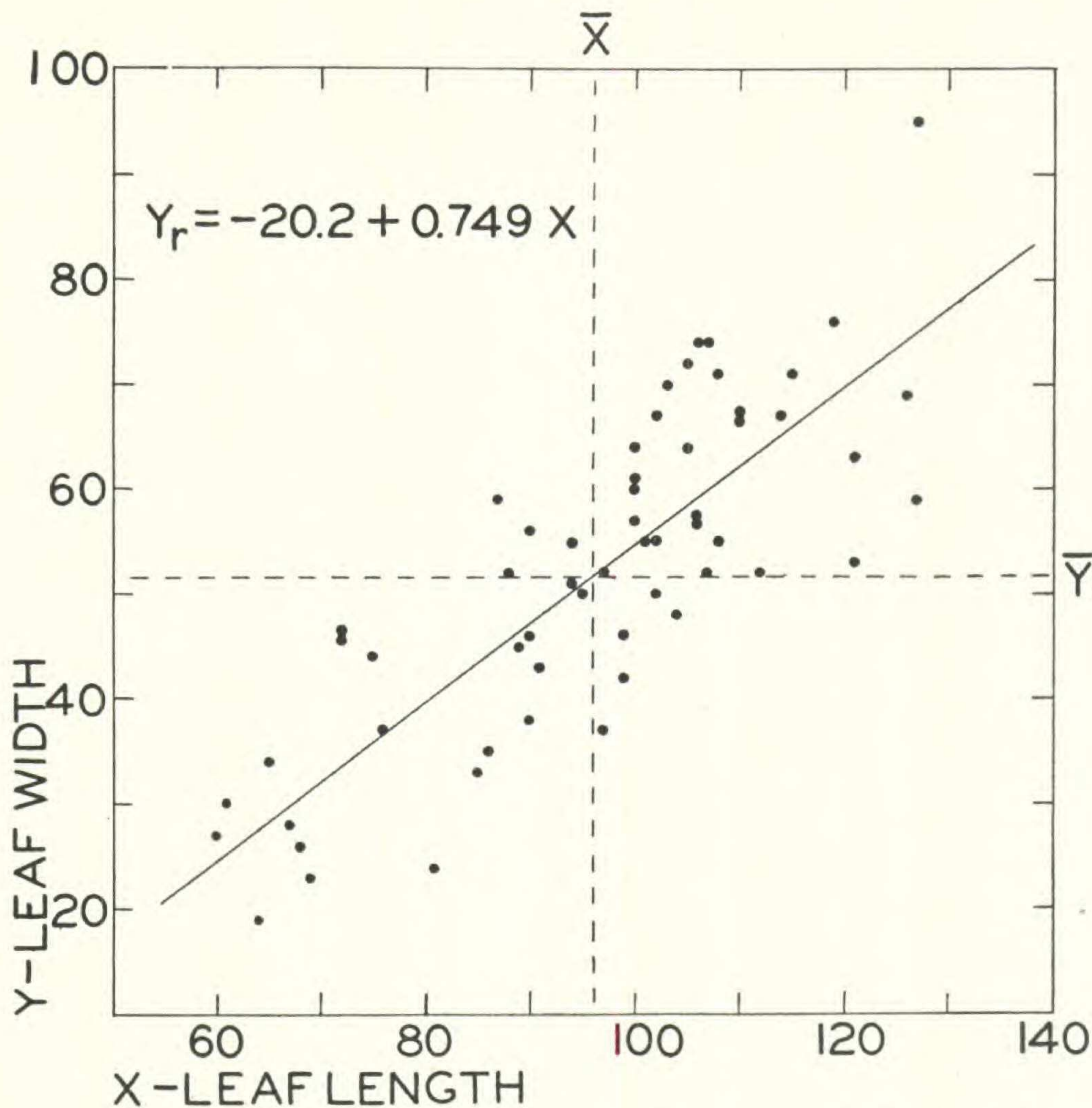


Fig. 17. Length and width measurements of 58 leaves of *C. Fremontii* var. *RiehlII* collected in 40-ft. quadrat no. 8 of fig. 1, B (R.6E, T.39N, S.4-0). Line of regression of width on length has been fitted to the data.

the mean length and width for the quadrat in which the leaf was collected (Table VIII, A, "within quadrats" rows), the sums of squares of deviations of the mean length and width for each quadrat from the over-all mean length and width ("among quadrats" rows), and the sums of squares of deviations of the length and width of each leaf from the over-all means ("total" row). Corresponding sums of products of the length and width deviations have also been determined. By the usual methods of analysis of variance, it is found that there is significantly greater variance among 20-ft. quadrats, 40-ft. quadrats, and 80-ft. quadrats, than within such quadrats, in both the length and width measurements (see the  $w$  and  $P$  values listed in Table VIII, A).

It is apparent in the field that the size of a leaf is greatly dependent on the general vigor of the plant. Presumably it is strongly influenced by environmental factors, and measurements of absolute length and width cannot be considered of much value in investigating the possibility of genetic differentiation from one portion of a glade to another. It might be supposed that the shape of a leaf is less strongly influenced by environmental variables. It is then of interest to investigate whether these data yield any information about local differentiation in



shape. One aspect of leaf shape is the relationship between width and length. One way in which this relationship might be expressed for a series of leaves is by the mean width:length ratio. When the width of each leaf is plotted against its length for a portion of the area studied, however, it appears that width and length are correlated, and that it may be justified to fit a regression line to the data (fig. 17). While the relationship between width and length may be regarded as approaching a linear one in the range of sizes at hand, the straight line fitted to the data does not pass through the origin. In other words, the width:length ratio does not tend to remain constant for leaves of varying length, but tends to increase with increasing leaf length.

Because of this circumstance, it is thought that the coefficient of linear regression of width on length is a more satisfactory index of the relationship between width and length than is the mean width:length ratio. It may be calculated by fitting an equation of the form  $Y_r = a + bX$  (where  $Y$  = leaf width,  $X$  = leaf length,  $a$  = intercept on the  $Y$ -axis and  $b$  = regression coefficient) to the data for a series of leaves by the method of least squares. This has been done for the data plotted in fig. 17. (The fact that the regression line does not pass through the origin, of course, indicates that it does not fit the data entirely adequately. The relationship between width and length is undoubtedly expressed properly by a curved line passing through the origin. Nevertheless, the coefficient of linear regression is regarded as adequate for the purposes of this statistical study.)

The problem of determining whether there is local differentiation in the relationship between leaf width and length from one portion of this area to another can then be restated as the statistical problem of determining whether the regression coefficients calculated for leaves from different portions of the area are significantly different. This could be done by calculating the coefficients, and applying Student's  $t$  test to the differences between pairs. It is possible to do this more efficiently, however, by carrying out an analysis of covariance.

The total variance in width has been divided above (Table VIII, A) into two portions, that within and that among quadrats. The analysis of covariance requires that it be subdivided further. It has been suggested above that there is a significant regression of width on length within at least one of the 40-ft. quadrats (fig. 17). The variance in width within each quadrat could then be subdivided into two portions: the variance of the regression line about the quadrat mean width, and the variance of the individual width measurements about the regression line. The sum of squares of deviations in width can then be written  $\Sigma (Y - \bar{Y})^2 = \Sigma (Y - Y_r)^2 + \Sigma (Y_r - \bar{Y})^2$  (where  $Y$  = width of an individual leaf,  $\bar{Y}$  = mean leaf width for a quadrat, and  $Y_r$  = theoretical width for a leaf calculated by substituting its length into the regression equation for the quadrat). If the length and width measurements for a quadrat are put in terms of deviations from the quadrat mean length and width, so that  $x = X - \bar{X}$  and  $y = Y - \bar{Y}$ , the three terms of this equation can be rewritten:  $\Sigma (Y - \bar{Y})^2 =$



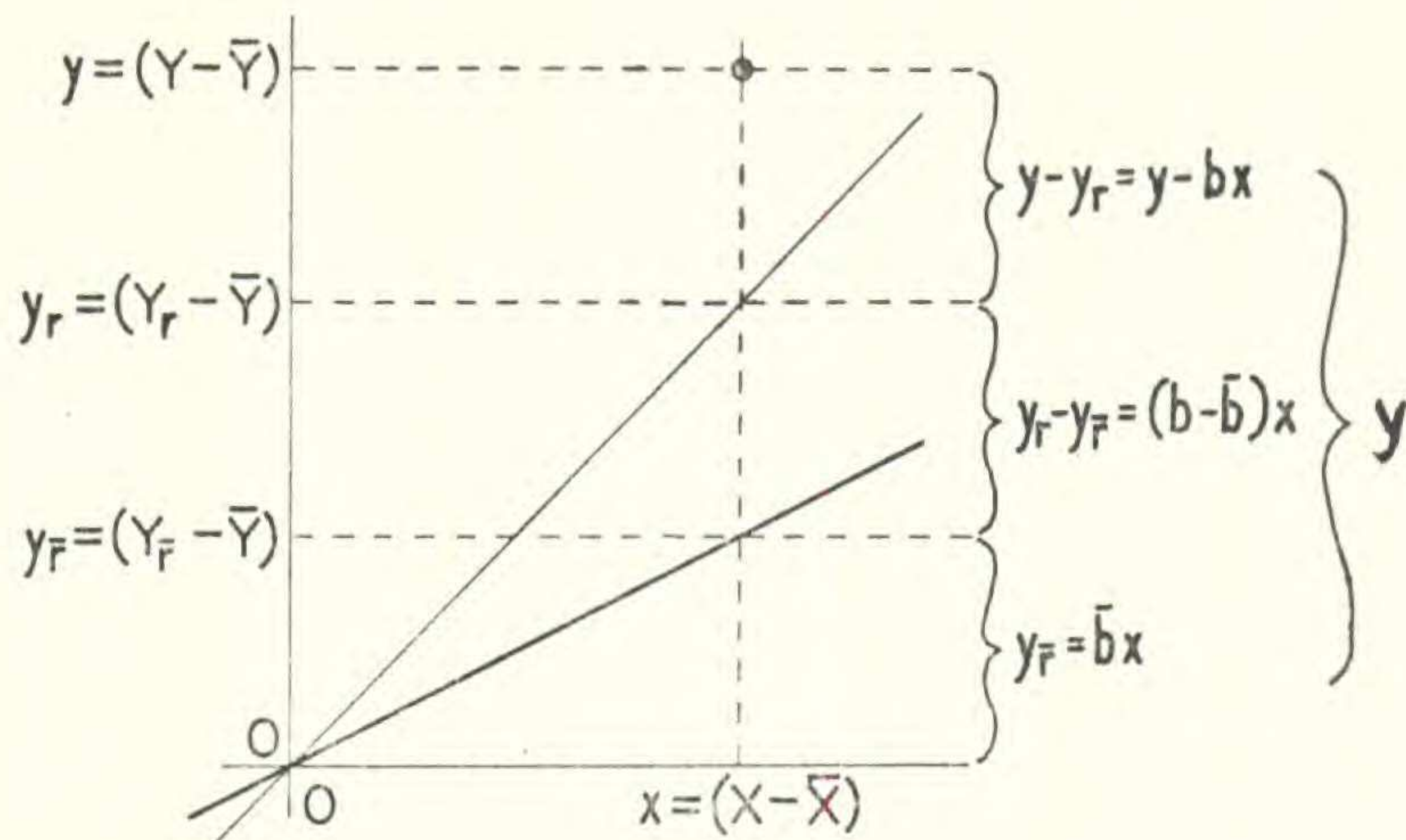


Fig. 18. Diagram to illustrate manner in which deviation in width of a leaf from its quadrat mean has been divided into three portions in the analysis of covariance. Further explanation in text.

$\Sigma y^2$ ,  $\Sigma (Y - Y_r)^2 = \Sigma y^2 - (\Sigma xy)^2 / \Sigma x^2$ , and  $\Sigma (Y_r - Y)^2 = (\Sigma xy)^2 / \Sigma x^2$ , as shown in any general treatise on statistics. The equation for the regression line can also be rewritten  $y_r = bx$  (where  $b = \Sigma xy / \Sigma x^2 =$  regression coefficient for the quadrat).

The regression line for a quadrat, whose equation is written in this way, passes through the origin, that is, through the point which corresponds with the quadrat mean length and width. The regression lines for each of the quadrats can then be visualized as radiating from a common origin. An average regression line for a series of quadrats, which passes through the same origin, can also be considered. Its equation can be written  $y_r = \bar{b}x$ . In fig. 18 are shown a regression line for one quadrat (lighter line) and the average regression line for a series of quadrats (heavier line). The deviation from the quadrat mean of a single measurement,  $y$ , can be seen to be made up of three portions: (1) the theoretical deviation from the quadrat mean, calculated by substituting the length deviation of the leaf into the average regression equation ( $y_r$ ); (2) the difference between the theoretical deviation calculated from the quadrat regression equation and that calculated from the average regression equation ( $y_r - y_r$ ); and (3) the difference between the actual width deviation and the theoretical deviation calculated from the quadrat regression equation ( $y - y_r$ ). It can be shown that the corresponding sums of squares of deviations in width for all the leaves over a series of quadrats are given by the following formulae:

(1) Sum of squares attributable to the average regression  $= (\Sigma \Sigma xy)^2 / \Sigma \Sigma x^2$ ;

(2) Sum of squares attributable to differences between the quadrat regressions and the average regression  $= \Sigma [(\Sigma xy)^2 / \Sigma x^2] - (\Sigma \Sigma xy)^2 / \Sigma \Sigma x^2$ ; and

(3) Sum of squares attributable to deviations of the separate width measurements from the regression within each quadrat, or "within-quadrats residuals"  $= \Sigma \Sigma y^2 - \Sigma [(\Sigma xy)^2 / \Sigma x^2]$ . (In each case, the first sign of summation,  $\Sigma$ , indicates summation over a series of quadrats, the second, summations for the series of



leaves within quadrats.)

By centering each of the quadrat regression lines at the origin, differences among the mean widths and lengths for each quadrat have been ignored. The variance in width arising from these differences has been shown to be significantly greater than that within quadrats (Table VIII, A), and it is of interest to subdivide it into two portions. The among-quadrats sum of squares of width deviations consists of:

(4) Sum of squares attributable to regression of the quadrat means, calculated most easily by substituting among-quadrats values from Table VIII, A, into an expression of the form  $(\Sigma xy)^2/\Sigma x^2$ ; and

(5) Sum of squares attributable to deviations of quadrat mean widths from their regression, or "among-quadrats residuals," obtained by subtracting (4) from the among-quadrats sum of squares of deviations in width.

If  $N$  is the total number of leaves over the area studied, and  $k$  the number of quadrats into which the area is divided, the number of degrees of freedom to be ascribed to each of the five sums of squares is: (1) average regression, 1; (2) regression differences,  $k - 1$ ; (3) within-quadrats residuals,  $N - 2k$ ; (4) regression of means, 1; (5) among-quadrats residuals,  $k - 2$ ; totalling to  $N - 1$ .

The within-quadrats residual mean square is to be regarded as the "error" mean square, with which other mean squares should be compared. The mean squares of interest in investigating leaf shape differences from one quadrat to another are: that attributable to regression differences (2), and the among-quadrats residual mean square (5). If the population were statistically uniform in the width to length relationship expressed by the regression coefficient, neither of these mean squares should be significantly greater than the within-quadrats residual mean square. (Dr. Donald R. Charles has given generously of his time in developing this scheme of analysis, in clarifying for the author the concepts involved, and in aiding in interpretation of the results of the analysis. The author, however, is responsible for this exposition of the method.)

The analysis of covariance of this leaf collection is summarized in Table VIII, B. A separate analysis has been made for each of the three schemes of subdivision of the area. It will be seen that the regression differences are not statistically significant from one 20-ft. or 40-ft. quadrat to another, but are significant among the 80-ft. quadrats. The among-quadrats residuals are significantly greater than the within-quadrats residuals from one 20-ft. or 40-ft. quadrat to another, but not among the three 80-ft. quadrats. In other words, there is statistically significant local differentiation in leaf shape from one portion of the area to another. This differentiation appears at the 20-ft. and 40-ft. levels of subdivision as significant deviations of the quadrat means from their regression, and at the 80-ft. level as differences among the within-quadrats regressions.

It may then be inquired whether this local differentiation in leaf shape, and in absolute length and width, follows any discernible pattern. For this purpose the outlines of the 40-ft. quadrats shown in fig. 1, B, have been redrawn (fig. 19). Within each quadrat outline have been placed the number of the quadrat ( $Q$ ),



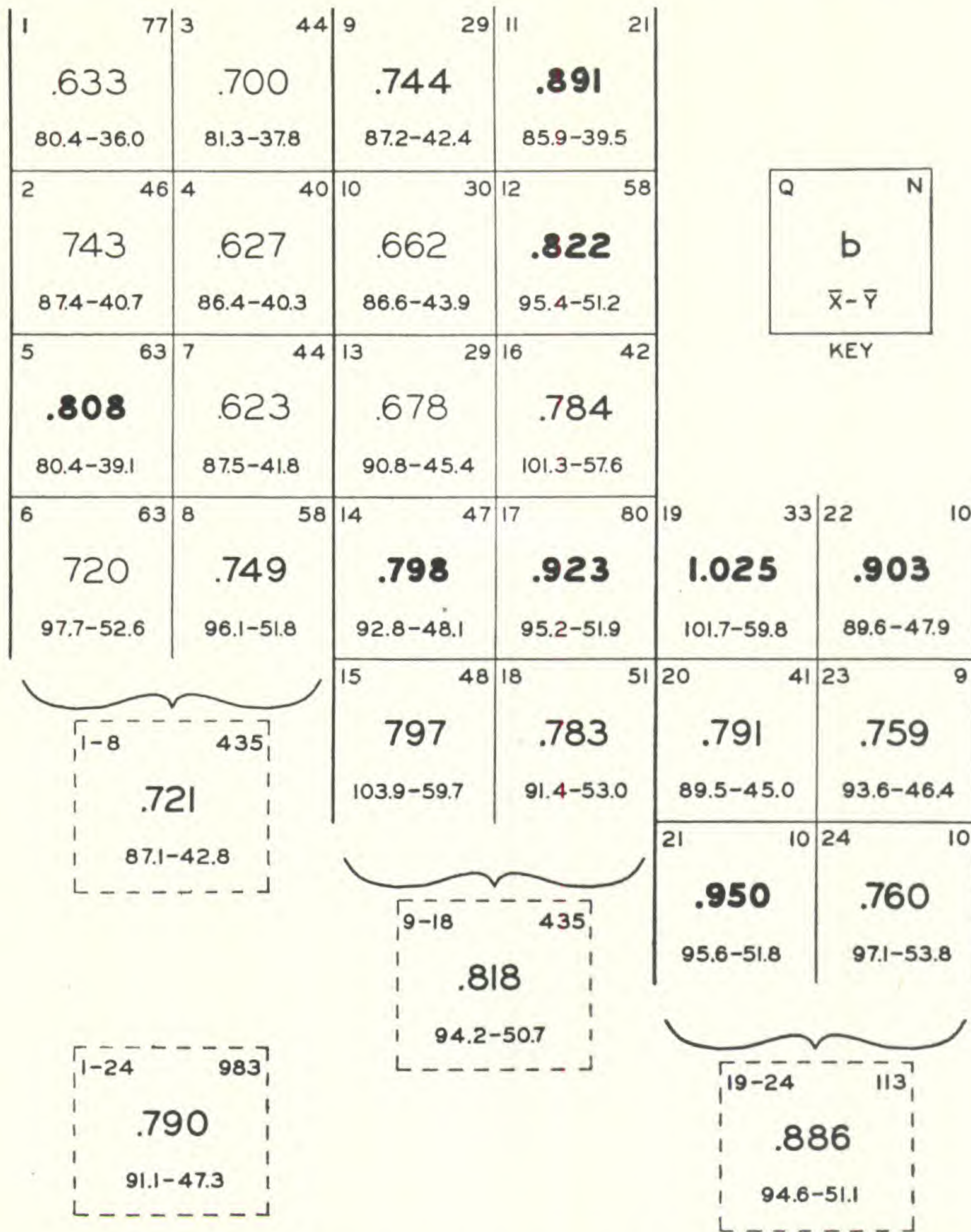


Fig. 19. Collection of leaves of *C. Fremontii* var. *Rieblii* made at R.6E, T.39N, S.4-0. Compare with fig. 1, B. Squares are 40-ft. quadrats. Number in center of each square is coefficient of regression of leaf width on length. Largest coefficients in heavy figures, intermediate coefficients in medium figures, and smallest ones in light figures. Further explanation in text.

the number of leaves measured from it (*N*), the regression coefficient of width on length for the leaves within the quadrat (*b*), and the mean length and width of the leaves ( $\bar{X}$  and  $\bar{Y}$ ). When the mean lengths and widths and the regression coefficients are compared, it is seen that the leaves of the upper-left portion of the diagram (embracing, perhaps, the nine quadrats numbered 1, 2, 3, 4, 5, 7, 9, 10 and 11, and a total of 394 leaves) are smaller and relatively narrower, than those from the remainder of the area. This grouping, however, is not without inconsistencies.



TABLE IX  
MEASUREMENTS OF LEAVES AT R. 2E, T. 42N, S. 15B  
Analysis of Variance and Covariance

A					
	$\Sigma x^2$	$\Sigma xy$	$\Sigma y^2$	Degrees of freedom	
Within transects	90,868.35	69,857.32	76,752.97	256	
Among transects	6,022.65	5,499.18	7,092.97	8	
Total	96,891.00	75,356.50	83,845.94	264	
For variance in length, $n_1 = 8$ , $w = 2.12$ , $P = 0.034$ For variance in width, $n_1 = 8$ , $w = 2.96$ , $P = 0.0036$					
B					
	Sum of squares of deviations	Degrees of freedom	Mean square deviation	$w$	$P$
Within transects:					
Average regression	53,704.56	1	53,704.56		
Regression differences	1,389.25	8	173.66	1.98	0.080
Residuals	21,659.16 (76,752.97)	247 (256)	87.69		
Among transects:					
Regression of means	5,021.13	1	5,021.13		
Residuals	2,071.84 (7,092.97)	7 (8)	295.98	3.38	0.0028
Total	83,845.94	264			

The second leaf collection which has been studied was obtained on a glade at R.2E, T.42N, S.15B (Glade no. 2 in fig. 2 and Table I). Nine transects, 10 ft. wide, were laid out at 250-ft. intervals across the glade, as illustrated in fig. 20. Collection of a leaf from each plant encountered yielded 265 leaves for measurement. The numbers for each transect range from 7 to 74. The analysis of variance and covariance described above was applied to these data also. There is significantly greater variance among transects than within in the absolute measurements of width, and perhaps of length (Table IX, A). The differences among the regressions of width on length for separate transects are not significant, but there are significant deviations of the transect means from the among-transects regression (Table IX, B). As before, this can be taken to indicate that there is statistically significant local differentiation in leaf shape from one portion of the glade to another.

In an attempt to determine whether the differentiation on this glade falls into any pattern, the transect regression coefficients have been entered near each transect in fig. 20. When the values of the coefficients are compared, it is seen that they can be arranged in two groups, those of transects 1, 3, 6, 8, and 9 being lower than those of transects 2, 4, 5 and 7. The leaves within each of these two groups of transects are statistically uniform in shape, as shown by an analysis of covariance applied to each. The glade can perhaps be thought of as divided into seven portions which have alternately wider and narrower leaves. These seven



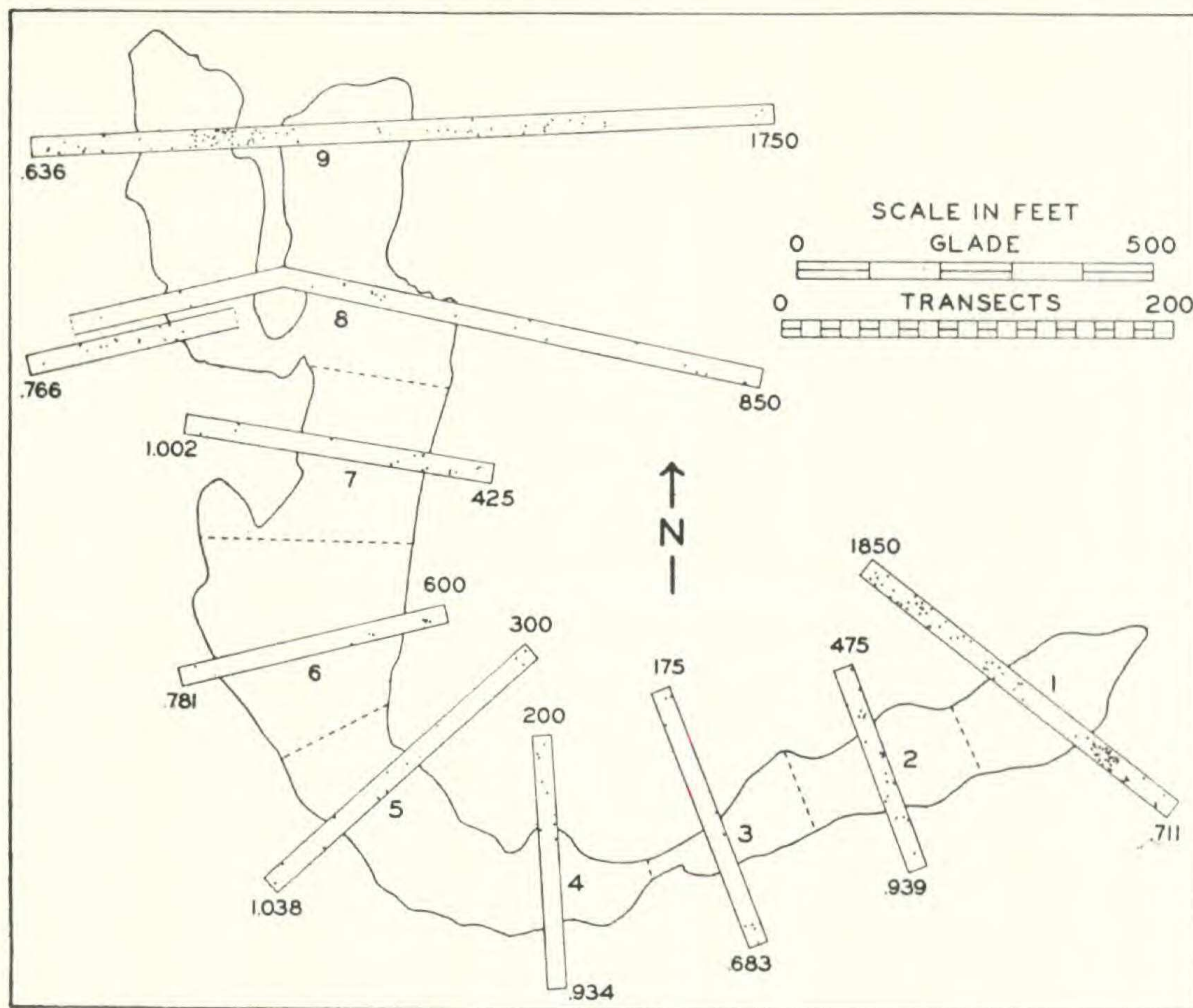


Fig. 20. Glade at R.2E, T.42N, S.15B, showing arrangement of nine transects, and distribution of plants (small dots) within transects. Length and width of a leaf from each plant have been measured. Transects have been enlarged for clarity. The fractional numbers near each transect are coefficients of regression of leaf width on length, the larger numbers have been obtained by multiplying the number of measured leaves from the transect by 25. Further explanation in text.

portions of the glade can perhaps be delimited by lines drawn midway between the transects, as has been done in fig. 20. A crude estimate of the number of plants represented by a transect is obtained by multiplying the number of leaves studied by 25, since the transects are 10 ft. wide and 250 ft. apart. These numbers have also been entered in fig. 20. If this picture of the pattern of differentiation is a true one, the differentiation appears to be effective between groups of plants numbering a few hundred.

An alternative picture of the differentiation pattern on this glade is to regard it as divided into three portions, embracing, respectively, transect 1, with an estimated 1850 plants ( $b = 0.711$ ), transects 2-7, with 2175 plants ( $b = 0.987$ ), and transects 8-9, with 2600 plants ( $b = 0.676$ ). In favor of this grouping is the fact that an analysis of covariance for transects 2-7 shows no significant differentiation in shape among the five transects. If this is the true picture, the differentiation would appear to be effective between groups of plants numbering about two thousand. It does not appear possible from these data to make a choice between the two alternatives.

No information about the factors responsible for the local differentiation in



leaf size and shape can, of course, be obtained from these data. The differentiation may be merely a result of differences in environmental conditions from one portion of a glade to another, or it may have a genetic basis. The glades are not believed to be a uniform habitat for *Clematis*. This topic has been discussed on pp. 418 and 419. This non-uniformity of the glades may give rise to the local differentiation in leaf size and shape merely by inducing environmental fluctuations in a population which is essentially homogeneous genetically. On the other hand, the distribution of *Clematis* on a glade is characterized by significant aggregation (p. 419). The degree of isolation between aggregates may be such as to allow a certain amount of random genetic differentiation. It should be pointed out that, on at least one of the two glades studied, the differentiation in leaf size and shape appears to be effective over a radius including a few hundred plants. Other lines of evidence, such as the distribution pattern, and observations of pollination, have suggested an effective population size of a few hundred.

The question of an environmental, as opposed to a genetic, basis for the demonstrated local differentiation, can also be directed at the results of the analysis of flower measurements (Tables VI and VII). It appears much less probable that the differences in flower measurements from one glade or region to another are environmental fluctuations than that the leaf differences discussed above can be so accounted for. There appears to be greater variation in physical conditions from one portion to another of a single glade than between separate glades taken as wholes. Furthermore, there is undoubtedly a good deal of truth to the systematists' principle that flower and fruit characters are more "stable" than are the characters of vegetative organs such as leaves. Subjective study in the field of variation within and among clones leads one to believe that the principle holds for differences among plants as well as for differences among species.

A number of subjective observations of variation have been made, which consistently point to the existence of a considerable amount of local differentiation. The glade at R.3E, T.42N, S.31D (Glade no. 3 in fig. 15) is small and relatively isolated. It supports 75 to 100 plants. The flowers strike one immediately by their lack of color and unusual proportions. The sepals are longer (one of them measured 51 mm.), and exceed the stamens much more than usual. Perhaps one-third of the plants share these characteristics, and, in other respects as well, show a resemblance which suggests close relationship. At R.2E, T.42N, S.18H (Glade no. 2 in fig. 13) a fairly large glade with a population of 1,000 or 2,000, 15 or so plants were seen which resemble each other in that the sepals are rolled back so as to expose about half the length of the stamen mass. This peculiarity was subsequently seen on a near-by glade, but has not been noticed elsewhere. Two monstrous plants, in which the leaves are irregularly coalesced and incised, and otherwise distorted, were seen among the estimated 600 plants at R.5E, T.40N, S.13A. No teratological specimens have been seen elsewhere, though an occasional ternate shoot has been found in the midst of a decussate-leaved clone. Exploration of the large glade at R.4E, T.41N, S.2F (Glade no. 5, fig. 13)



disclosed only eight plants in a small area at one end. The six which were in flower at the time of the visit were remarkably alike in flower color and form and in general habit. The resemblance suggested that of sibs rather than of portions of a clone. Similar "family resemblances" of plants which are growing fairly close together have been seen less distinctly in many other instances.

Examination of the aerial photograph tracings and field experience both indicate that the glades are, on the whole, smaller and more isolated in the Robertsville region at the northwestern end of the range than they are farther southeast. The difference is more pronounced than the distribution map (fig. 13) suggests. An impression has grown during the field work that this difference in size and degree of isolation of separate colonies is reflected in a difference in the degree of variability in different parts of the range. The plants of the Robertsville region strike one as displaying more variation in the amount of color and pattern of color distribution in the sepals; in the width, texture, and degree of crimping of the expanded margin of the sepal; in the size, shape, and general aspect of the leaves. In general, there is a larger proportion of "queer-looking" plants than among the more uniform population of the Platin region.

#### SOURCES OF VARIATION

Gene mutation is generally regarded as the ultimate source of evolutionary change, and it would be desirable in studying the evolution of any organism to begin with information about the rate and direction of mutation of its genes. However, such information has been obtained for relatively few genes in a very few organisms which are favorable genetic material. Needless to say, no data whatever on this point are available for *Clematis*, and the plant is not favorable material for genetic study, because of the long period required before it reaches flowering age.

Chromosomal changes such as ploidy, inversion, and translocation have been demonstrated to be responsible for evolutionary change in several organisms. Polyploidy is practically non-existent in the genus *Clematis*. All the reported species are normal diploids ( $n = 8$ ), with the exception of two tetraploid cultivated forms (Meurman and Therman, '39, Gregory, '41). The author has found the haploid number,  $n = 8$ , in several plants of *C. Fremontii* var. *RiehlII*. Examination in the field of the first division of the microsporocytes of about 75 plants has disclosed no chromatin bridges; in these plants at least, there were no conspicuous inversions.

Hybridization between species and varieties of higher plants is of rather frequent occurrence, and appears to be an important factor in the evolution of many forms. Anderson and Hubricht ('38) have studied a case of introgressive hybridization between two species of *Tradescantia*. Mangelsdorf and Reeves ('39) regard probable hybridization with *Tripsacum* as an important factor in the evolution of maize. Wide crosses are known to occur in the genus *Clematis*. *C. integrifolia*, which is fairly closely related to *C. Fremontii* var. *RiehlII*, has given



rise to *C. Durandi* by a cross with *C. Jackmani*, one of the large-flowered oriental hybrids. A hybrid of *C. integrifolia* with *C. Flammula*, one of the small-, paniculate-flowered species, is also known, and instances of hybridization between other species can be multiplied (Rehder, '40). The fact, then, that *C. Pitcheri*, which is a member of the same section (VIORNA) of the genus as *C. Fremontii* var. *Rieblii*, occurs in the vicinity of the glades makes hybridization between the two species seem at least a possibility. Transfer of pollen between the two species appears possible but must be a rare occurrence. *C. Pitcheri* often occurs in the woods just below a glade, and bumblebees, at least, visit both species (Robertson, '28). However, they are separated by a difference in flowering period. *C. Fremontii* var. *Rieblii* has finished flowering by the second week of May, and *C. Pitcheri* does not come into flower until the middle of June. It continues to flower for some time, and it would probably be in anthesis when *C. Fremontii* var. *Rieblii* flowers sporadically in September.

Five plants have been found which strongly suggest that hybridization does occur. One of the plants (fig. 21, fig. 22, C) appears to be the  $F_1$  progeny of a cross between the two forms. It grows on a glade at R.2E, T.42N, S.14H, about 3.4 mi. southeast of Robertsville. A graded farm-to-market road, surfaced with gravel, crosses the lower edge of this large glade. The supposed hybrid is rooted in the gravel embankment at the down-slope side of the road. The glade above the road is well populated with *C. Fremontii* var. *Rieblii*, a few plants persisting in the gravel at the edges of the road. A number of rather small plants of *C. Pitcheri* occur in the 300-ft. strip of woods between the road and Little Calvey Creek. The site of the hybrid is suggestive, since other species hybrids have often been reported to occur in disturbed habitats.

On the basis of morphological characters, it is impossible to regard the presumed hybrid as a member either of *C. Fremontii* var. *Rieblii* or of *C. Pitcheri*, variable as the latter species is. It appears to show pronounced hybrid vigor. Making allowances for that, it appears roughly intermediate between the two parental forms in the characters which have been examined. It has the ascending habit of *C. Pitcheri*, but the stems are considerably stouter. It appears intermediate in degree of compounding of the leaves between *C. Fremontii* var. *Rieblii*, with simple leaves, and *C. Pitcheri*, whose leaves are compound or decomposed, though this character is difficult to evaluate. Its leaflets appear as thick and coriaceous as the leaves of *C. Fremontii* var. *Rieblii*, contrasting with the much thinner leaflets of *C. Pitcheri*. In *C. Fremontii* var. *Rieblii* the flowers are solitary, terminating the vegetative branches; in *C. Pitcheri* single flowers are borne on axillary peduncles, each with one pair of simple floral leaves. In the supposed hybrid both conditions occur (fig. 21). The flowers are intermediate in size between those of the putative parents. The sepals are less recurved, and their valvate margins narrower, than in *C. Fremontii* var. *Rieblii*; in these two characters the plant approaches *C. Pitcheri*. Its flowering period is a week or two later than that of *C. Fremontii* var. *Rieblii*, and earlier than that of *C. Pitcheri*. The clusters of





Fig. 21. Plant which is presumed to be the  $F_1$  progeny of a cross between *C. Fremontii* var. *RiehlII* and *C. Pitcheri*. Note the old flower terminating the primary stem from which sepals and stamens have fallen. Scale in centimeters.



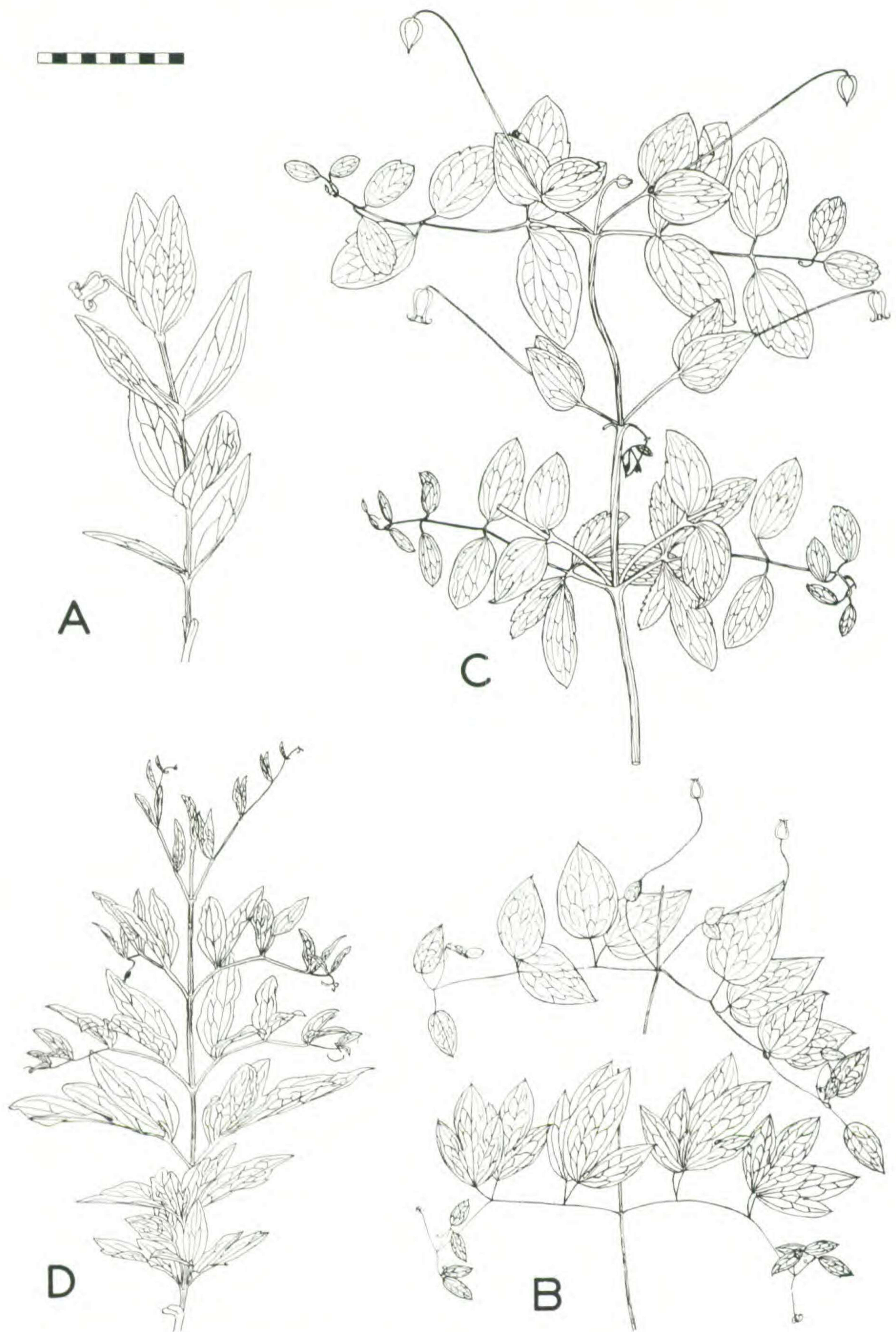


Fig. 22. *C. Fremontii* var. *Rieblii*, *C. Pitcheri* and two plants which are presumed to be the result of hybridization between them. A. Tracing of a photograph of a young plant of *C. Fremontii* var. *Rieblii*. B. Tracing of two pressed fragments of a large plant of *C. Pitcheri*. C. Tracing of a portion of a pressed plant, which is presumed to be the  $F_1$  hybrid between *C. Fremontii* var. *Rieblii* and *C. Pitcheri*. Another shoot of the same clone has been illustrated in fig. 21. D. Tracing of a photograph of a plant which is presumed to have resulted from back-crossing of the  $F_1$  to *C. Fremontii* var. *Rieblii*. Scale in centimeters.



achenes, and the achenes themselves, are larger than in either of the supposed parents. The achene-tails are naked as in both *C. Fremontii* var. *Rieblii* and *C. Pitcheri*.

It has not been possible to compare the plant with a *C. Fremontii* var. *Rieblii* × *C. Pitcheri* hybrid of known parentage.

The evidence at hand indicates that the supposed hybrid is fertile, though the crucial test of germinating the seeds has not been made. Several full heads of achenes have been seen, and the seeds appear viable on examination. The pollen appears normal in the microspore stage. Five microsporocytes at metaphase I have been analyzed completely. In each of them there appear to be eight normal bivalents. A larger number of cells at this stage have been examined more briefly, and all appear normal. Chromatin bridges have been seen in two microsporocytes out of about 50 at late anaphase I. These observations suggest that there may be one or more inversions differentiating the parents of the supposed hybrid, but that pairing is sufficiently normal to allow formation of good seed.

The supposition that the hybrid between *C. Fremontii* var. *Rieblii* and *C. Pitcheri* is fertile, is consistent with finding of the four other aberrant plants. They all resemble *C. Fremontii* var. *Rieblii* more closely than does the supposed  $F_1$  plant discussed above, but are clearly outside the normal limits of variability of the former. They are not uniform among themselves, and can be arranged in a series according to the degree in which they resemble *C. Fremontii* var. *Rieblii*. Of these four plants, one found at R.2E, T.42N, S.7A and one at R.2E, T.42N, S.15B resemble the  $F_1$  most closely. Next in order is a second plant found at R.2E, T.42N, S.7A (fig. 22, D), and the plant found at R.3E, T.41N, S.1D is nearest to *C. Fremontii* var. *Rieblii*. They suggest a series of backcrosses of the  $F_1$  to *C. Fremontii* var. *Rieblii*.

If these suppositions are correct, some introgression of *C. Pitcheri* genes into the *C. Fremontii* var. *Rieblii* population presumably occurs. It is estimated that the number of plants which have been seen at close enough range to detect such aberrant forms as the five described above is of the order of 10,000. These figures indicate, to a first approximation, the frequency of the presumed introgression. The five plants discussed above appear to have a considerable amount of *C. Pitcheri* germ-plasm; it might also be expected that a larger number of plants would exhibit the presence of a smaller amount. It is not known, of course, in what way small amounts of *C. Pitcheri* germ-plasm might be evidenced. The most striking difference between the two species is the contrast between the simple leaves of *C. Fremontii* var. *Rieblii* and the compound leaves of *C. Pitcheri*. Occasionally a plant is seen which departs from the norm in a coarse tothing of the larger leaves, which are usually entire. It may be that this is evidence of some *C. Pitcheri* genes.

It might also be expected that introgression occurs in the converse direction. The fact that *C. Pitcheri*, *C. Fremontii*, and *C. Fremontii* var. *Rieblii* are the only plants in the genus lacking plumose achene tails is suggestive of exchange of genes between the species over a long period of time.



Another possible source of variability deserves mention. The data on frequency of colored sepal tips (Table IV), the measurements of sepal characters (Table VII), and subjective field observations lead to the conclusion that the plants of the Robertsville region diverge more greatly from the norm for the entire population than do those of the other three regions. The Robertsville glades as a group are relatively isolated, as can be seen by reference to one of the maps, (*e. g.*, fig. 2). It may be that this relative isolation is sufficient to account for the singularity of the Robertsville plants. One is inclined, however, to speculate on the possibility that the population of *C. Fremontii* var. *Rieblii*, limited as it is, may at one time have consisted of two smaller groups. One would suppose that the two groups were centered near Plattin and near Robertsville, since *Clematis* appears most abundant in these regions at present. Their merger may have taken place rather recently, in view of the presumed increase in numbers since white settlement of the Ozarks. This possibility has great evolutionary importance. If the population were at one time divided into two wholly isolated groups, considerable divergence between them would presumably have occurred. The hybridization resulting from their reunion would provide a source of variation of greater magnitude than that provided by gene mutation governed by the statistical mechanism which Wright hypothesizes, and of somewhat different nature than that provided by introgression of *C. Pitcheri* genes.

The variation which is seen in this *Clematis* population could well be the resultant of these three factors. Introgressive hybridization with *C. Pitcheri* is likely. It probably does not occur with great frequency, but genes of adaptive value in the glade habitat may occasionally be introduced into the population by this means. Isolation of the Robertsville region has allowed it to evolve to some extent along its own course, whether one considers the partial isolation of the present, or the possibly complete isolation of some past period. The supposition that some random differentiation of partially isolated groups of plants on separate glades or portions of glades occurs by the mechanism which Wright has described, is consistent with the pattern of distribution of the plant, and with the statistical pattern of variation in several morphological characters. Since it is probable that the effective population size is comparatively large, the fate of individual genes is probably not wholly a random matter, but is under some selective control.

The concept of this *Clematis* population which emerges is not that of an "old" endemic in which evolutionary change has ceased and which is doomed to extinction, though its restricted range may suggest such a picture to some minds. It is rather that of a population which has undergone marked changes in range and in numbers, and which appears to be increasing in numbers at present; one in which evolutionary changes of several sorts are occurring, though perhaps not as rapidly as in many organisms. *C. Fremontii* var. *Rieblii*, because of its presumably low competitive vigor, is probably doomed to restriction to the glade habitat. Its breeding structure is neither that of approximate panmixia which leads to extreme



specialization, nor of extreme restriction in numbers which leads to wholly non-adaptive differentiation. This being so, it may be expected to continue to thrive on the glades, and perhaps to extend its range, though the colonization of new glades will probably be slow.

#### SUMMARY

*Clematis Fremontii* var. *RiehlII*, which is wholly restricted to dolomitic barrens, or glades, in an area of about 400 sq. mi. in east-central Missouri, has been studied in the field with particular attention to features of its distribution, biology, and pattern of variation, which are of evolutionary importance.

The population, estimated at 1,500,000, is organized into a hierarchy of natural subdivisions: *regions* of glade concentration; *clusters* of glades; *colonies* of the plant, which correspond approximately with glades; and *aggregates* of a very few, to perhaps a thousand, plants on each glade. There is great inequality in number of plants from one *colony* or *aggregate* to another. Both types of subdivision exhibit partial isolation, of a degree which is regarded as favorable for continuing evolution. The plant appears to be remarkably stable in numbers, but there is indirect evidence that it has increased since the white settlement of the Ozarks.

Inefficient seed dispersal and the longevity of the plants are factors which probably tend to promote a high degree of inbreeding. Counteracting them is the pollination of the plant by wide-ranging insects, which tends to promote cross-breeding.

Statistical study of morphological variation shows significant local differentiation at three levels of the distributional hierarchy: from one region to another, in five flower characters; from one glade to another, in four flower characters; and from one portion of a glade to another, in leaf shape. The most significant feature of the regional differentiation is the singularity of the plants near Robertsville, at the northwestern end of the range.

The pattern of distribution; the biological factors of pollination, seed dispersal and germination, and longevity; and the nature of variation in leaf shape are consistent in suggesting that the effective population size is a few hundred.

There is evidence that introgressive hybridization with *C. Pitcheri* occurs. This, together with differentiation on a regional scale, and local differentiation of a moderately random nature appear to be the most significant evolutionary processes occurring in the population.



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